AUG 18 1916
UNIV. OF MICH.
LIBRARY

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXI AUGUST, 1916 No. 2

CONTENTS

		PAGE
EWING, H. E.	*Eighty-seven Generations in a Parthenogenetic Pure Line of Aphis avenæ Fab*	53
WHITNEY, DAVID D.	*The Transformation of Brachionus pala into Brachionus amphiceros by Sodium Silicate*	113
GOLDSMITH, WM. M.	*Relation of True Nucleolus to the Linin Network in the Growth Period of Pselliodes cinctus*	121

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY
& SON
28 Essex Street, Strand
London, W. C.

AGENT FOR GERMANY
R. FRIEDLÄNDER
& SOHN
Berlin N. W.
Carlstrasse, 11

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter, under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

Jacques Loeb—*The Rockefeller Institute for Medical Research*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXI. *August, 1916.* *No. 2*

BIOLOGICAL BULLETIN

EIGHTY-SEVEN GENERATIONS IN A PARTHENOGENETIC PURE LINE OF APHIS AVENÆ FAB.

H. E. EWING.

The chief features of my work with *Aphis avenæ* Fab. are here summarized:

1. The pure line theory, as originally applied to self-fertilized plants or asexually reproducing plants and animals, was tested in eighty-seven generations of a parthenogenetic insect.

2. Selections were made for forty-four consecutive generations in an attempt to modify a character that is known to be easily modified by selection in higher animals that reproduce sexually.

3. Variations, both continuous and abrupt, were studied in regard to their transmissibility.

4. The occurrence of pædogenesis is here recorded.

5. The effect of continued parthenogenetic reproduction on the virility and metabolism of a strain has been determined.

6. The effects of temperature on growth, size, reproduction, and dimorphism are here noted.

Introductory Considerations.

About three years ago when the writer began his studies in pure lines, it appeared that the time was quite opportune for some one to carry on breeding experiments with some parthenogenetic insect. Up to that time no one had tested the pure line theory of inheritance in any of the higher arthropods. After attempting breeding work, first with our common cabbage aphis, *Aphis brassicae* Linn., and later with the black cherry aphis, *Myzus cerasi* Fab., I finally started the experiments with *Aphis avenæ* Fab. which are here reported. A very few of the results

obtained with this species have been published,[1] but none of those obtained beyond the first fifteen generations. Here there is given for the first time a complete report of the pure line work with *Aphis avenæ* Fab. up to the last generation obtained, the eighty-seventh. I regret that, at the end of the eighty-seventh generation, excessive heat killed all the individuals of the line; stopping further experimentation which might have given results of even more importance.

Plant lice are especially well adapted for experimental purposes in pure lines. They belong to the order Hemiptera, a group of insects showing a relatively high degree of organization. They can be reared easily on many small succulent plants; are so small that a score or more of their breeding cages can be kept in a small laboratory, yet they are large enough to be easily handled and mounted on microscope slides. Plant lice reproduce very rapidly, and give a sufficiently large number of progeny. The species used gave a new generation every six days with an average number of progeny of from twelve to twenty individuals. *Aphis avenæ* Fab. is in fact so well adapted for pure line work in another respect that it was easily made the choice of the species available in the locality where the experiments were started. This characteristic is its ability to reproduce continually asexually, or parthenogenetically, under the natural conditions found on the Pacific slope—the place where the work was begun. Occasionally sexual forms have been reported from these milder portions of the United States, but never has the present writer found them there. In the north central and in the northeastern states the sexual forms are common as in the case of other species of aphids. The choice of this species has been, I believe, very fortunate, as the results here given will attest.

But to begin with what are our conceptions as to the effect of selection in a pure line? They are simply these: Selection in a pure line of self-fertilized plants or animals, or plants or animals reproducing asexually, no matter for how long carried on does not change the somatic characters that are dependent upon the germplasm. These somatic characters, according to the theory,

[1] "Pure Line Inheritance and Parthenogenesis," BIOL. BULL., Vol. XXVI., pp. 25–35; and, "Notes on Regression in a Pure Line of Plant Lice," *l. c.*, Vol. XXVII., pp. 164–168. (Both published in 1914.)

will unfold themselves generation after generation—within a certain range of variability—in constant conformity with an ancestral type. This type Johannsen terms the genotype. It is simply the type of the race. Theoretically, then, the germplasm is as unchangeable as adamant, and selection with all its rigors should in the end leave the race exactly as it was in regard to its inheritable qualities.

In recent years the term pure line has been applied by some workers[1] to the pure strains of cross-fertilized plants or animals, but incorrectly so, I believe. Johannsen did not consider it as so applying; neither did Jennings, or many of the other workers coming after them. Undoubtedly the theory as originally propounded was not construed as applying to cross-fertilized plants or to sexually-reproducing animals. There is no such thing as purity in Johannsen's sense where the melting-pot of amphimixis alloys the germplasm of one individual with the germplasm of another. In 1911 he stated that we had shown, "to excess that phenotypes may seem totally 'pure' and nevertheless be heterogeneous." He further remarks: "Thus constancy as to the phenotype of the progeny is no sure proof for genotypical purity or unity." Indeed how else could it be if we are to assume the frequent presence of multiple unit characters, and thus accept the infinity-factor hypothesis now constantly put forward by Mendelians to explain what appears to be blending in the case of certain hybrid crosses. This distinction becomes doubly important when we consider the confusion and possible causal relation between heterozygosity expressing itself in segregation of characters, even minute, and the appearance of the so-called mutants. It is in relation to mutation, that the pure line advocates have, in the opinion of the present writer, need to be alarmed at the application of the term pure line to a supposedly homozygous pure strain of cross-fertilized organisms.

The Effectiveness of Selection in a Parthenogenetic Pure Line.

In order to test the effectiveness of selection in a parthenogenetic pure line several different sub-lines were bred in isolation.

[1] Pearl for example. See Pearl, 1915, "Seventeen Years Selection of a Character Showing Sex-linked Mendelian Inheritance," *Amer. Nat.*, Vol. XLIX., pp. 595–608.

These sublines I will refer to as isolated sublines, or simply isolations. In each of these, selections were carried on for a few or for many generations to see if there was produced any inheritable effect, due to the picking out of individuals showing extreme variation in the character considered.

Selections Made in an Attempt to Change the Ratio of the Length of the Third Segment of the Antenna to the Fourth Segment. Previous Results. Isolations Nos. 1 and 2.

The first character used for observing the effects of selection was that of the ratio existing between the lengths of the third and fourth segments of the antenna. For fifteen generations selections were made in an attempt to change the mean of this ratio for the line, which was found to be 1.80 to 1, *i. e.*, it was found that on the average that the third segment was 1.80 times as long as the fourth segment. Selections were made for the first 10 generations in the attempt to increase this ratio. At the end of the tenth generation the fraternal mean was 1.66 to 1, or decidedly less than the mean for the pure line. Selection had here failed utterly to produce any inheritable effects. Various other selections were made, and the results of the selections with this character have been reported in the two short papers previously referred to. In the writer's conclusion, published in the first of these papers, the following statement was made which summarizes the results so far as the effects of selection are concerned in changing the ratio of the length of the third to the fourth segment of the antenna: "Selections from among extreme variants do not alter the mean as obtained for the strain without selection. The fraternal mean of any generation may show a great fluctuation from the mean of the strain, but this fluctuation is not transmitted to following generations."

Selections Made in an Attempt to Increase the Length of the Cornicles. Isolation No. 3.

After making selections for fifteen generations using the ratio existing between the length of the third and fourth segments of the antennæ as a character, much time was spent in studying the specimens obtained for characters that were sufficiently variable,

and at the same time suitable in other respects for use in further selection work. It was noticed that the cornicles (honey tubes) were quite variable both in size and shape. Also it was observed that they were fairly well chitinized, and that they did not appreciably shrink while being passed through killing and clearing fluids in preparation for mounting on microscope slides and further that when mounted on microscope slides they could easily be measured. At first I thought that I would take the ratio existing between the length of the cornicles and the length of the body as a suitable character. Then it was noticed that there was some shrinkage of the body due to the action of the killing and clearing fluids, which, at the time, I feared would cause an introduction of serious errors in measurements. For this reason I finally decided to make selections for the purpose of attempting to increase, not relatively, but absolutely, the length of the cornicles. Such selections, it is observed, should not only tend to increase the length of the cornicles in relation to the length of the body, but also to increase the size of the individuals in the line. In other words, two distinct characters are involved in the consideration of variations in the absolute cornicle-length.

Before going into the details of the selections made in this isolation (isolation No. 3) it is probably best that a few words as to methods be given. In all cases the individuals of a fraternity were treated alike, they were placed in the same killing and clearing fluids, and each individual was kept in each fluid about the same length of time. The specimens were all mounted in balsam on microscope slides, and measurements were made with an ocular micrometer. In each instance the individual whose cornicles were to be measured was allowed to produce its progeny before being killed. If it was the one which was to be selected for carrying on the strain its progeny were saved, isolated while yet immature, and reared until they each gave individuals of the next generation. This method of allowing each individual to rear its progeny before being killed (in order to examine the characters) being the one used throughout the experiments here reported. I find that it is one that commends itself for several reasons, among them the following:

(*a*) Characters can be much better observed, and measured

more accurately in dead, mounted specimens than in live individuals.

(*b*) The fecundity of the individual is known when the individual is selected, thus obviating the termination of the experiment through the unsuspected sterility or low fecundity of the selected individual.

(*c*) Permanent mounts of all individuals examined are desirable as a permanent record to be used later in collecting valuable data.

While making selections in isolation No. 3, I decided upon a system of numbering individuals which was used throughout the series of experiments. According to this system each individual is given in reality three numbers which are written together as one. The first of these, which is written much larger than the second, refers to the number of the isolation (*i. e.*, the isolated subline of the main pure line). The second number, which is written much smaller (a subnumber), refers to the generation to which the individual belongs counting from the stem agamic mother that was first isolated from individuals found in nature. The third number, written the same size as the first, refers to the individual's number in the fraternity to which it belongs. Thus 3_{16} 5, written together as a single number for an individual means that this individual belongs to isolation 3 (the isolation here considered), generation 16, and has been given the number 5 as its individual fraternity number to designate it from the other members of the 3_{16} fraternity.

Before starting the selections in this isolation the mean cornicle-length for the line was determined by getting the mean for all the individuals obtained in six previous generations. The mean length of the cornicles of the wingless agamic females was found to be 2.609. The mean length for the winged females was found to be 2.441. Or in other words the cornicles of the wingless agamic females were found to be 1.065 times longer than the cornicles of the winged agamic females. During the process of selection several winged forms were met with. In such instances the measurements of their cornicles were reduced to the denominator of the wingless form simply by multiplying by 1.065.

Selections in isolation 3 were begun in the 15th generation and continued for 11 generations, ending in the 26th. In almost

every instance the individual selected for carrying on the strain had the longest cornicles of any in its fraternity, and in all instances its cornicles were much longer than the mean obtained for the fraternity. The fraternal means obtained run as follows[1]

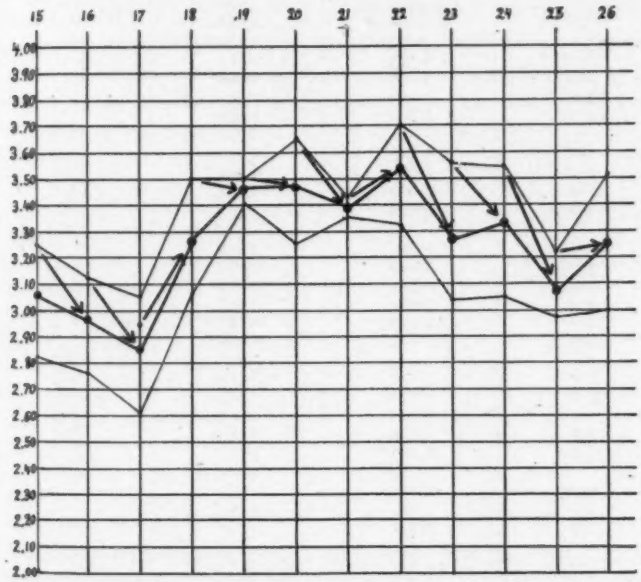


FIG. 1. Graph, or curve, showing the results of selections made in isolation No. 3 in an attempt to increase the length of the cornicles. The heavy middle line joining the large black dots shows the fluctuations of the fraternal means. The light lines show the fluctuations of the extremes of the different fraternities. The arrows show the amount of regression or digression in each case.

beginning with the mean for the 3_{15} generation and fraternity: 3.058; 2.979; 2.847; 3.260; 3.463; 3.475; 3.387; 3.510; 3.276; 3.321; 3.083; 3.250. The more complete data obtained for isolation 3 are found in tabular form in Table I.[2]

[1] In the selection work in this isolation an artificial standard of measurement was employed throughout. The number refers to the divisions of the eye micrometer, and they were not reduced to the metric scale. In the subsequent isolations all measurements have been reduced to millimeters, unless specifically stated otherwise.

[2] The computations involved in the preparation of the data in this table and in those which follow were made for the first time by the writer, and were also made later by a student in order to get a check on the results.

TABLE I.

SHOWING MEASUREMENTS OF CORNICLES OF INDIVIDUALS OBTAINED IN ISOLATION 3, AND COMPUTATIONS OF THE FRATERNAL MEANS (AVERAGE CORNICLE LENGTH FOR EACH FRATERNITY).

Individual.	Average Length of Cornicles.	Winged Forms Corrected.	Fraternal Means.
3n1	3.175		
3n2	2.675	2.828	
3n3	3.250		
3n5	2.800	2.980	
			3.058
3n2	3.025		
3n3	2.600	2.769	
3n4	3.000		
3n5	3.125		
			2.979
3n1	2.450	2.609	
3n2	2.875	3.061	
3n3	2.550	2.715	
3n4	2.950		
3n5	2.575	2.740	
3n7	2.825	3.008	
			2.847
3n1	3.200		
3n2	3.075		
3n5	3.500		
3n6	3.175		
3n11	3.350		
			3.260
3n2	3.400		
3n3	3.275	3.490	
3n6	3.500		
			3.391
3n2	3.500		
3n3	3.550		
3n4	3.350		
3n5	3.400		
3n6	3.250		
3n8	3.450		
3n9	3.650		
3n10	3.525		
3n11	3.550		
3n12	3.525		
			3.475
3n1	3.350		
3n4	3.425		
			3.387
3n1	3.500		
3n2	3.525		
3n3	3.325		
3n5	3.500		
3n7	3.700		
			3.510
3n1	3.350	3.570	
3n2	2.975	3.050	
3n3	3.200	3.400	
3n5	3.150	3.350	

TABLE I.—*Continued.*

Individual.	Average Length of Cornicles.	Winged Forms Corrected.	Fraternal Means.
3n6	3.250		
3n7	3.000	3.200	
3n8	2.850	3.040	
3n9	3.100	3.300	
3n10	3.200	3.400	
3n11	3.000	3.200	
			3.276
3n1	3.450		
3n3	3.050		
3n4	3.225		
3n6	3.475		
3n8	3.300		
3n9	3.550		
3n12	3.200		
			3.321
3n3	2.800	2.980	
3n6	3.025		
3n7	2.925	3.110	
3n8	3.025	3.220	
			3.083
3n1	3.250		
3n2	3.200		
3n3	3.475		
3n5	3.200		
3n6	3.100		
3n7	3.525		
3n8	3.000		
			3.250

We note that there is a great fluctuation in the fraternal means obtained. During the early selections, disregarding these fluctuations, the general trend appears to be the augmenting of the fraternal means. But during the latter part of the experiment there is a decided drop in the means, and the last one obtained, 3.250, is much below the mean for the whole line. Hence we fail to find in the continuous selection for 11 generations that any permanent effects were produced. But why the great rise and fall in the fraternal means? This I attribute to the great variation in the size of the individuals obtained, which variations were closely correlated with the variations in the absolute cornicle length. The size variation was undoubtedly influenced by the change in temperatures, as I succeeded in demonstrating later. The results of this demonstration will be given beyond in this paper. Selection, therefore, failed to influence in the least the mean length of the cornicles in the pure line of *Aphis avenæ* Fab.

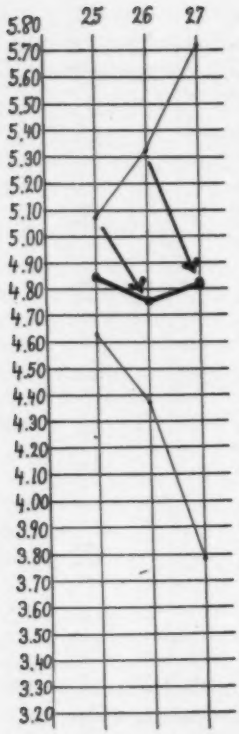


FIG. 2. Plot showing the results of selection in isolation No. 4, for long narrow cornicles. The figures in a column to the left represent the lengths of the cornicles in terms of their widths which in each case is taken as unity. The generations are numbered at the top. Curve plotted as in Fig. 1.

graphically in Fig. 2. are given in Table II.

Selections Made for Long Narrow Cornicles. Isolation No. 4.

In this isolation selections were made in an attempt to increase the length of the cornicles in comparison with their width at their distal ends (at the cornicle-ring). Selections for only three generations were made. The first selection was made in the twenty-fifth generation. The ratio expressing the mean for this fraternity was 4.86 (*i. e.*, on the average the cornicles of this fraternity were 4.86 times as long as broad at their distal ends). The individual with the highest ratio, $4_{25}1$, was selected from this fraternity. It gave 13 offspring that were reared to maturity. The mean for these 13 offspring (the twenty-sixth generation) was found to be 4.77. This mean is slightly lower than the one for the previous generation. From the twenty-sixth generation, the individual with the highest ratio, $4_{26}3$, was selected for obtaining the next generation. It gave the twenty-seventh generation, producing 6 adults with a mean ratio of 4.82, or four hundredths less than that of the mean of the fraternity from which the first selection was made.

We find then that no effects of selection are evident in this isolation. The results obtained for isolation 4 are shown The measurements and ratios obtained

TABLE II.

SHOWING MEASUREMENTS OF CORNICLES OF INDIVIDUALS OBTAINED IN ISOLATION 4, AND COMPUTATIONS OF THE FRATERNAL MEANS (THE AVERAGE RATIO OF THE CORNICLE LENGTHS TO WIDTHS FOR ALL MEMBERS OF A FRATERNITY).

Individual.	Average Length of Cornicles.	Average Width of Cornicles.	Ratio of Length to Width.	Fraternal Means.
4II1	47.00	9.25	5.08	
4II2	47.50	9.75	4.87	
4II3	44.00	9.50	4.63	
				4.86
4III	47.00	9.50	4.95	
4II2	48.00	10.00	4.80	
4II3	49.25	9.25	5.32	
4II5	48.50	10.00	4.85	
4II6	48.50	10.00	4.85	
4II7	47.00	10.75	4.37	
4II8	50.00	10.50	4.76	
4II9	47.00	9.50	4.95	
4II10	49.25	11.00	4.48	
4II11	44.50	9.50	4.68	
4II12	49.00	11.00	4.45	
4II13	47.25	9.50	4.97	
4II14	49.25	10.75	4.58	
				4.77
4II1	45.00	8.00	5.62	
4II2	47.25	10.50	4.50	
4II3	49.50	10.50	4.71	
4II4	45.50	12.00	3.79	
4II6	54.25	9.50	5.71	
4II8	50.50	11.00	4.59	
				4.82

Selection Made for Increasing the Body Length,—Isolation No. 5; and also Selections Made in a Check Isolation (No. 6) for Decreasing the Body Length.

Because stature, or body length, is one of the most commonly studied of the fluctuating variations, and also because it is a character well known to be inherited in some of our higher animals, I decided to see if by continued selection in a pure line it could be affected. Experiences with isolation No. 3, having taught me that size was influenced easily by temperature changes and probably by other changes also, I decided to eliminate errors from these sources by running a check isolation, or subline. And in order to test more rigorously the effects of selection, in this check strain the shortest individuals were picked out in each instance. Selections were begun in the twenty-sixth generation in each isolated subline, and continued until the thirty-second

generation was obtained. The means obtained for each subline are here written in parallel rows (the measurements being given in millimeters), the two belonging to the same generation being superimposed:

Isolation 5—1.582; 1.909; 1.890; 1.603; 1.135; 1.600.

Isolation 6—1.640; 1.628; 1.763; 1.488; 1.390; 1.180.

Here it is observed that two of the six means obtained in the check isolation 6, in which isolation selections were made from the shortest individuals are actually greater than those for the corresponding generations in isolation 5 where selections were

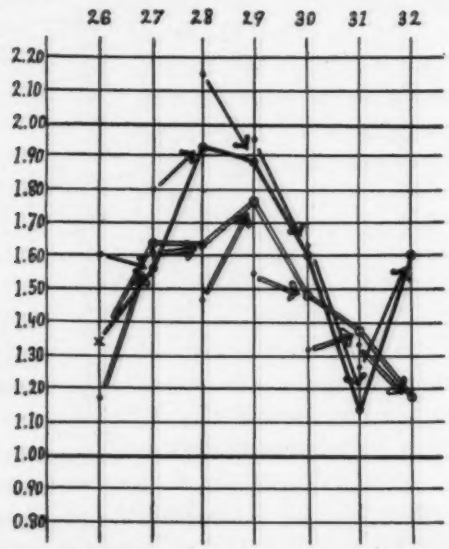


FIG. 3. Plotted curves for check isolations Nos. 5 and 6. In isolation No. 5 (curve represented by heavy line) selections were made for increasing the body length. In isolation No. 6 (curve represented by light open line) which was run as a check to 5, selections were made for decreasing the body length. Graph made on same plan as in previous figures except that fluctuations of extremes are not given.

made from the longest individuals. Further, if we plot the curves of the means obtained for the two sublines together (see Fig. 3), we find that they go up and down together and cross at three different points. The fluctuations in size were found to

TABLE III.

SHOWING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 5 AND CHECK ISOLATION 6, AND THE COMPUTED FRATERNAL MEANS FOR BOTH ISOLATIONS.

Isolation No. 5.			Isolation No. 6.		
Individual.	Length in Millimeters.	Fraternal Mean	Individual.	Length in Millimeters.	Fraternal Mean.
5201	1.15				
5202	1.48				
5203	1.60				
5205	1.18				
5206	1.24				
5207	1.47				
5208	1.17				
		1.327			
5272	1.80		6271	1.67	
5273	1.47		6272	1.61	
5274	1.65				
5275	1.62				
5276	1.37				
		1.582			1.640
5281	1.91		6281	1.81	
5282	1.64		6283	1.65	
5283	2.07		6284	1.85	
5284	1.97		6285	1.32	
5285	1.75		6286	1.67	
5286	1.98		6287	1.47	
5287	1.97				
5288	2.15				
5289	1.88				
52810	1.77				
		1.909			1.628
5291	1.85		6291	1.81	
5292	1.94		6292	1.82	
5293	1.95		6293	1.77	
5294	1.84		6294	1.78	
5295	1.87		6295	1.90	
			6296	1.84	
			6297	1.75	
			6298	1.48	
			6299	1.55	
			62910	1.74	
			62911	1.77	
			62912	1.95	
		1.890			1.763
5301	1.31		6302	1.72	
5302	1.71		6303	1.32	
5303	1.61		6304	1.64	
5304	1.80		6305	1.34	
5305	1.57		6306	1.54	
5306	1.62		6307	1.52	
			6308	1.38	
			6309	1.44	
			63010	1.50	
		1.603			1.488

TABLE III.—*Continued.*

Isolation No. 5.			Isolation No. 6.		
Individual.	Length in Millimeters.	Fraternal Mean.	Individual.	Length in Millimeters.	Fraternal Mean
5n1	1.27		6n1	1.34	
5n2	1.00		6n2	1.45	
			6n5	1.38	
		1.135			1.390
5n2	1.60		6n1	1.30	
			6n2	1.06	
		1.600			1.180

be about as great as before (in isolation 3), but here they *went together*, and were not apparently affected by selection. In the case of isolation No. 5, the final fraternal mean obtained was exactly the same as the length of the first individual selected in this subline. In the case of isolation No. 6, the final mean obtained is found to be just one hundredth of a millimeter more than the length of the first individual selected. The results of the selections in these two check sublines are presented graphically in Fig. 3, and the measurements and some other data obtained are given in Table III.

The results obtained for these six generations show no effects of selection as far as size is concerned. What are the effects of selection on other variable characters?

Selections Made for Increasing the Length of the Antennæ in Comparison with the Length of the Body. Isolation No. 7.

It was found that there was a variation not only in the length of the antennæ in comparison with the length of the body, but also a great variation in the absolute length of the antennæ of the different individuals of a fraternity. Since the latter variation involves a complex character, the former was thought to be best adapted for use in selection tests. In isolation No. 7 selections were made in an attempt to increase the length of the antennæ in comparison with the length of the body. They were carried on for five generations. In every case the individual with the longest antennæ in comparison with its body length was selected. The means (which express the ratios existing between the antennal lengths and body lengths) obtained are as

follows, beginning with the twenty-ninth generation:[1] 1:1.257, 1:1.278, 1:1.162, 1:1.178, 1:1.320.

In two instances the fraternal means show the antennal lengths to be greater than they were for the first fraternity obtained. The last fraternal mean obtained, 1:1.320, shows the average

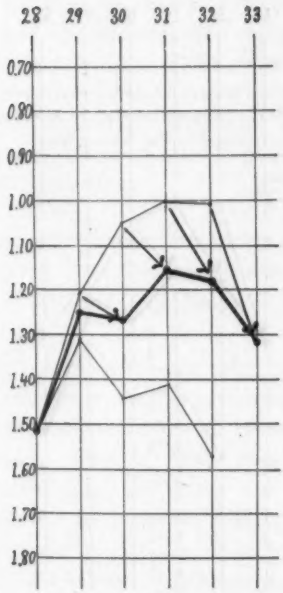


FIG. 4.

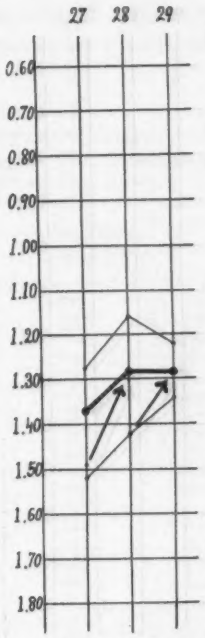


FIG. 5.

FIG. 4. Plotted curve for isolation No. 7. Selections made for increasing the length of the antennæ in comparison with the length of the body. The figures in a column to the left refer to the length of body in terms of the antennal length of the same individual taken as unity. Curve plotted on same plan as in previous figures.

FIG. 5. Results of selections made in isolation No. 8, in the opposite direction from those made in isolation No. 7. Curve plotted on same plan as in Fig. 4.

length of the antennæ in comparison with that of the body to be considerably less than it was in the first fraternity (twenty-ninth generation) obtained, but it was considerably longer than

[1] The length of the antennæ is taken as unity as it is always less than the length of the body.

the antennæ of the parent individual of this fraternity. The stem parent of this isolation, or subline, had a very high antennal ratio, however.

The results of selection in this line are well represented graphically in Fig. 4, which shows the relations of the fraternal means to each other, and to the extreme variants of the different fraternities. Further data in regard to the individuals obtained in isolation 7 are found in Table IV.

TABLE IV.

SHOWING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 7, AVERAGE LENGTHS OF THEIR ANTENNÆ, THE RATIO OF BODY LENGTH TO ANTENNAL LENGTH, AND THE COMPUTED RATIO FOR THE FRATERNAL MEAN.

Individual.	Length of Body, Artificial Standard of Measure.	Ave. Antennal Length, Artificial Standard of Measure.	Ratio.	Fraternal Mean.
7s1	114	87.0	1.31	
7s2	117	90.0	1.30	
7s6	121	100.0	1.21	
7s17	112	92.0	1.22	
				1.257
7s3	132	106.5	1.24	
7s4	131	103.5	1.27	
7s6	135	93.5	1.44	
7s9	135	97.5	1.38	
7s13	132	101.0	1.31	
7s14	123	116.0	1.06	
				1.278
7n1	102	101.5	1.00	
7n2	115	98.0	1.17	
7n3	96	93.0	1.03	
7n4	98	96.5	1.02	
7n5	119	97.0	1.23	
7n6	123	90.5	1.36	
7n7	126	89.0	1.42	
7n8	117	102.0	1.15	
7n9	126	99.5	1.27	
7n10	104	96.0	1.08	
7n12	109	96.5	1.13	
				1.162
7s1	95	77.5	1.23	
7s2	105	82.0	1.28	
7s3	100	77.5	1.29	
7s4	82	78.5	1.04	
7s6	75	71.5	1.05	
				1.280
7s2	95	72.0	1.32	

Again, we fail to see any hereditary influence shown in regard to a fluctuating character, or variation. Further tests were made.

Selections Made for Shortening the Length of the Antennæ in Comparison with that of the Body. Isolation No. 8.

In isolation No. 8 selection was made in the reverse direction from that of isolation No. 7, using the same character, *i. e.*, the length of the antennæ in comparison with the length of the body. Only two selections were made, but the fraternal means for both generations showed that the average length of the antennæ was greater than in the fraternity from which the first selection was made, thus showing no inheritance of this fluctuating character. The results for isolation 8 are further explained in Fig. 5 and Table V.

Selections Made in an Attempt to Increase the Length of the Cornicles in Comparison with that of the Body. Isolation No. 9.

When selections were made in isolation No. 3 to increase the absolute length of the cornicles, two variable characters were involved. One of these was a partial variation, and the other an individual variation. In isolation No. 9, selections were made in attempting to increase the length of the cornicles in comparison with the length of the body, a simple partial variable. Only three selections were made in this case which gave the following means (which show the ratio of the cornicle-lengths in percentage terms of the body lengths): 21.12 per cent., 16.86 per cent., and 21.09 per cent. The first and the last of these means are considerably above the ratio for the stem progenitor of the subline which was 18.8 per cent. The second fraternal mean was much below the ratio for this stem progenitor.

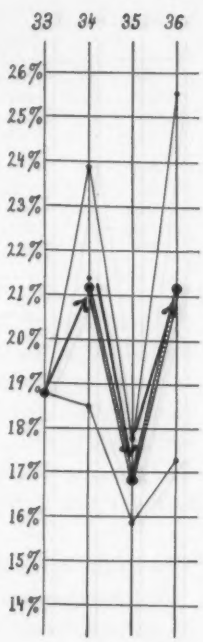


FIG. 6. . Plot showing results of selection in isolation No. 9, where an attempt was made to increase the length of the cornicles in comparison with that of the body. The lengths of the cornicles expressed in percentage terms of the body length are found in the column of figures to the left.

TABLE V.

GIVING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 8, THE AVERAGE LENGTHS OF THEIR ANTENNÆ, THE RATIO OF BODY LENGTH TO ANTENNAL LENGTH, AND THE RATIO REPRESENTING THE FRATERNAL MEAN.

Individual.	Length of Body Artificial Standard of Measure.	Average Antennal Length, Artificial Standard of Measure.	Ratio.	Fraternal Mean.
8_{113}	121	94.0	1.29	
8_{114}	136	95.5	1.42	
8_{115}	121	103.5	1.17	
8_{117}	123	94.0	1.31	
				1.290
8_{112}	127	98.0	1.30	
8_{113}	116	87.0	1.33	
8_{114}	126	95.5	1.32	
8_{115}	127	94.5	1.34	
8_{116}	120	97.5	1.23	
				1.298

The results of the selections in this isolation are in themselves too meager to show anything, but taken together with the results obtained in previous isolated sublines they simply furnish additional proof that selection in a parthenogenetic pure line does not affect hereditarily any of the ordinary individual variations. The meager results obtained for isolation 9 are shown more fully in Fig. 6 and Table VI.

The Effects of Continuous Selection for Forty-four Generations on a Character that is Known to be Easily Modified by Selection in Animals that Reproduce Sexually.

All the selections thus far reported were not continued over a very large number of generations, and the results obtained with them may not be convincing to some of our adherents to the belief that selection is effective when made from fluctuating variations and continued for a large number of generations. But if selections are continued for a much longer period do we discover any appreciable effects? In order to give a more thorough test to the effectiveness of selection in a pure line, I decided to make selections for a great many generations using a character that is well known to be inherited in higher animals and plants where cross fertilization takes place. Few characters have been more thoroughly studied or more generally employed in selection

TABLE VI.

GIVING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 9, THEIR AVERAGE CORNICLE LENGTH, THE LENGTH OF THE CORNICLES IN PERCENTAGE TERMS OF BODY LENGTH, AND THE COMPUTED PERCENTAGES REPRESENTING THE FRATERNAL MEAN.

Individual.	Length of Body Artificial Standard of Measure.	Average Antennal Length, Artificial Standard of Measure.	Length of Cornicles in Percentage Terms of Body Length.	Fraternal Mean.
9n1	112.0	20.75	18.5	
9n3	102.5	21.00	20.5	
9n5	91.0	21.75	23.9	
9n7	102.0	21.50	21.1	
9n8	93.0	20.00	21.6	
9n9	106.0	22.75	21.5	
				21.12
9n2	104.0	18.50	17.8	
9n3	103.0	17.50	17.0	
9n5	118.0	18.75	15.9	
9n6	124.0	21.50	17.5	
9n8	125.0	21.50	17.2	
				16.86
9n1	112.0	22.50	20.1	
9n2	119.0	20.50	17.2	
9n4	115.0	22.75	19.8	
9n6	95.0	20.50	21.6	
9n7	108.0	22.00	20.4	
9n8	100.0	22.25	22.2	
9n11	96.0	23.00	23.9	
9n12	90.0	23.00	25.5	
9n14	102.0	22.50	22.1	
9n16	118.0	21.25	18.0	
				21.09

work than that of stature, or body length. It was this character that was used by Galton when he formulated his law of regression, and it is a character that is well known to be inherited under the influence of amphimixis. Furthermore, body length is a character the variation of which can be easily measured, and by past experience had proved to be a suitable one to work with for various reasons; hence this one was decided upon.

In isolation 11 selections were made only from among wingless agamic females, in fact after a few generations had been obtained I learned that by controlling somewhat the temperature only such forms appeared. When selections were started in isolation 11 it had been planned to continue them for a hundred generations; and, if by the time the twentieth generation had been obtained no effect of selection had appeared, to change the conditions of the experiment for each subsequent score of generations

except that the selection should continue for increasing the body length, or stature. The reason for doing this was to study the effects of temperature and food upon the character under consideration, especially to see if changing environmental conditions should stimulate any germplasm change. In all 44 generations were obtained. The first score of generations were treated in a definite way, the conditions were considerably changed for the second score of generations, and again changed for the last four generations.

First Score of Generations; Temperature not Regulated; Growth and Reproduction Period for Each Generation Not Made Uniform.

For the first score of generations the aphids were reared on young wheat shoots, in large glass vials which were used as breeding cells. They were placed on these young wheat shoots before the same were more than two or three days old. Usually these wheat shoots were less than twenty-four hours old when the lice were placed on them. Each louse was placed on the tender white sheath of the shoot just above the soil. It was sometimes found that after the shoots became a little older and had put out their first leaf that the plant louse would migrate upward and get on this leaf. When such a thing occurred the aphid was always removed to the sheath of the shoot down next to the soil. The breeding cells containing the young wheat shoots and plant lice were kept in the laboratory under such conditions as were found to prevail there. There was a great variation in temperature and a considerable variation in humidity, but Headlee has shown[1] that the latter does not affect the metabolism of aphids when the variations are not greater than would ordinarily occur under laboratory conditions. Further, no definite time was allowed during the first 20 generations for the growth and reproduction period of each fraternity. Individuals were allowed to reach maturity and produce from several to as many as two dozen offspring; then the parent individuals were killed, and measured and selection was made.

Selections in the first score of generations of isolation 11 were

[1] Headlee, T. J., 1914. "Some Data on the Effect of Temperature and Moisture on the Rate of Insect Metabolism," *Jour. Econom. Ent.*, Vol. VII., pp. 413–417.

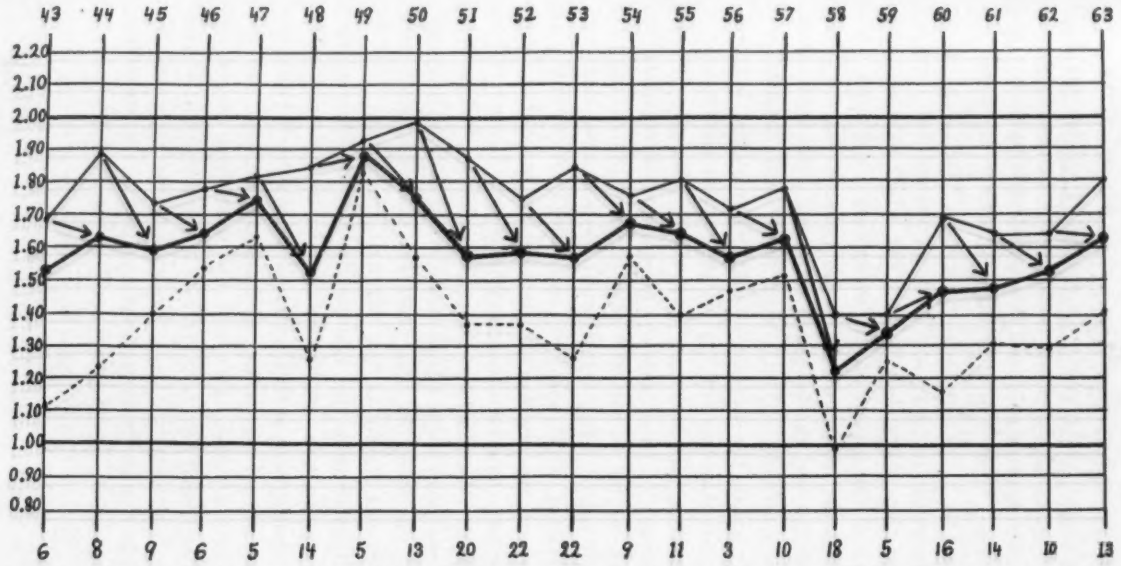


FIG. 7. Plotted results of selections for the first score of generations in isolation No. 11 for increasing the body length. The heavy solid line represents the fluctuations of the fraternal means; the light solid line the fluctuations of the longest variants; the broken line the fluctuation of the shortest variants. The amount of regression is indicated by the arrows. Column of figures to left, length of individuals in millimeters. Numbers at top represent the generations; at the bottom, the number of individuals measured in each fraternity.

begun in the forty-third generation, and at the end of the sixty-third generation a total number of 239 adult, wingless individuals had been obtained. In every instance the longest individual was selected from among those of the same fraternity for carrying on the subline. The results of these selections are given graphically in Fig. 7, and in tabular form in Table VII. If we examine the graph, or curve, in Fig. 7, we observe that there is a great fluctuation of the heavy solid line which represents the fluctuations of the means of the succeeding fraternities. The highest mean is 1.882 mm., the mean obtained for the forty-ninth fraternity and the lowest is 1.212 mm., the mean of the fifty-eighth fraternity. The longest individual obtained was 1.99 mm. in length, the shortest obtained was 0.99 mm. in length, or slightly less than one half that of the longest individual.

Looking along the curve, or line which joins the different fraternal means of these 20 generations, and disregarding the fluctuations, we fail to find any general trend upward, and the final mean obtained, 1.617 mm., is but little above the mean with which we started, which was 1.525 mm.; and the next to the last mean obtained, the one for the sixty-second generation, 1.527 mm., is within only two thousandths of a millimeter of the original mean. We conclude, then, that selection had no effect in shifting the mean of the line or strain, which is what Johannsen would call the mean for the *genotype*. But why such great variation in the means of the different fraternities? In the forty-eighth generation and again in the fifty-eighth generation we find a sudden and remarkable drop in the fraternal mean. I observed that in both of these cases the laboratory became quite cold, in fact so cold that it was very uncomfortable to stay in it for any length of time. Hence at the time these means were obtained I supposed that they were so unusually low on account of the low temperature. Later I demonstrated experimentally that a great change in temperature caused a correspondingly great change in the size (hence length) of the individuals.

TABLE VII.

GIVING LENGTH MEASUREMENTS OF INDIVIDUALS OBTAINED IN THE FIRST SCORE OF GENERATIONS OF ISOLATION 11, AND THE COMPUTED FRATERNAL MEANS. THE FEW WINGED FORMS THAT APPEARED WERE NOT MEASURED, AND ARE NOT GIVEN IN THIS TABLE.

Individual	Length in Millimeters	Fraternal Mean.
II₁₁1	1.56	
II₁₁2	1.59	
II₁₁3	1.69	
II₁₁4	1.56	
II₁₁5	1.61	
II₁₁9	1.14	
		1.525
II₁₄1	1.81	
II₁₄3	1.23	
II₁₄4	1.31	
II₁₄5	1.73	
II₁₄6	1.69	
II₁₄7	1.51	
II₁₄8	1.80	
II₁₄10	1.89	
		1.621
II₁₆1	1.64	
II₁₆2	1.69	
II₁₆3	1.69	
II₁₆4	1.66	
II₁₆5	1.40	
II₁₆6	1.43	
II₁₆7	1.49	
II₁₆8	1.61	
II₁₆10	1.73	
		1.593
II₁₈1	1.57	
II₁₈2	1.61	
II₁₈3	1.77	
II₁₈5	1.61	
II₁₈7	1.73	
II₁₈9	1.53	
		1.637
II₁₇1	1.63	
II₁₇3	1.70	
II₁₇4	1.81	
II₁₇5	1.76	
II₁₇10	1.80	
		1.740
II₁₈1	1.33	
II₁₈2	1.47	
II₁₈3	1.56	
II₁₈4	1.39	
II₁₈5	1.84	
II₁₈6	1.33	

TABLE VII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
$II_{48}8$	1.81	
$II_{48}9$	1.63	
$II_{48}10$	1.69	
$II_{48}11$	1.66	
$II_{48}12$	1.30	
$II_{48}13$	1.31	
$II_{48}14$	1.59	
$II_{48}15$	1.26	
		1.512
$II_{49}4$	1.87	
$II_{49}7$	1.91	
$II_{49}8$	1.87	
$II_{49}9$	1.83	
$II_{49}11$	1.93	
		1.882
$II_{50}1$	1.99	
$II_{50}2$	1.61	
$II_{50}3$	1.83	
$II_{50}4$	1.67	
$II_{50}5$	1.69	
$II_{50}6$	1.57	
$II_{50}7$	1.71	
$II_{50}8$	1.83	
$II_{50}10$	1.63	
$II_{50}11$	1.77	
$II_{50}12$	1.64	
$II_{50}13$	1.97	
$II_{50}14$	1.74	
		1.742
$II_{51}1$	1.66	
$II_{51}2$	1.41	
$II_{51}3$	1.57	
$II_{51}4$	1.56	
$II_{51}5$	1.66	
$II_{51}6$	1.64	
$II_{51}7$	1.40	
$II_{51}8$	1.64	
$II_{51}9$	1.53	
$II_{51}10$	1.59	
$II_{51}11$	1.63	
$II_{51}12$	1.87	
$II_{51}13$	1.57	
$II_{51}14$	1.56	
$II_{51}15$	1.61	
$II_{51}16$	1.63	
$II_{51}17$	1.77	
$II_{51}18$	1.37	

TABLE VII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
II$_{11}$19	1.41	
II$_{11}$20	1.53	
		1.580
II$_{11}$2	1.63	
II$_{11}$3	1.66	
II$_{11}$4	1.43	
II$_{11}$5	1.37	
II$_{11}$6	1.59	
II$_{11}$7	1.59	
II$_{11}$8	1.60	
II$_{11}$9	1.49	
II$_{11}$10	1.74	
II$_{11}$11	1.61	
II$_{11}$12	1.40	
II$_{11}$14	1.71	
II$_{11}$15	1.70	
II$_{11}$17	1.49	
II$_{11}$18	1.64	
II$_{11}$19	1.41	
II$_{11}$20	1.64	
II$_{11}$21	1.73	
II$_{11}$22	1.61	
II$_{11}$24	1.63	
II$_{11}$25	1.70	
II$_{11}$26	1.60	
		1.590
II$_{11}$1	1.59	
II$_{11}$2	1.57	
II$_{11}$3	1.69	
II$_{11}$4	1.70	
II$_{11}$5	1.74	
II$_{11}$7	1.34	
II$_{11}$8	1.84	
II$_{11}$9	1.73	
II$_{11}$10	1.51	
II$_{11}$11	1.50	
II$_{11}$12	1.63	
II$_{11}$13	1.63	
II$_{11}$14	1.64	
II$_{11}$15	1.40	
II$_{11}$16	1.47	
II$_{11}$17	1.41	
II$_{11}$18	1.26	
II$_{11}$19	1.50	
II$_{11}$20	1.51	
II$_{11}$21	1.46	
II$_{11}$24	1.76	
II$_{11}$25	1.43	
		1.560

TABLE VII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
II$_{142}$	1.74	
II$_{143}$	1.76	
II$_{144}$	1.63	
II$_{145}$	1.66	
II$_{146}$	1.69	
II$_{147}$	1.64	
II$_{148}$	1.61	
II$_{149}$	1.74	
II$_{14}$II	1.56	
II$_{154}$	1.67	1.670
II$_{156}$	1.60	
II$_{157}$	1.67	
II$_{159}$	1.40	
II$_{15}$II	1.61	
II$_{16}$I3	1.56	
II$_{16}$I8	1.57	
II$_{16}$I9	1.66	
II$_{162}$I	1.77	
II$_{162}$4	1.73	
II$_{162}$5	1.80	
II$_{168}$	1.71	1.640
II$_{16}$I4	1.47	
II$_{16}$I6	1.53	
II$_{17}$I	1.63	1.570
II$_{172}$	1.60	
II$_{173}$	1.51	
II$_{175}$	1.59	
II$_{179}$	1.73	
II$_{17}$II	1.77	
II$_{17}$I2	1.59	
II$_{17}$I3	1.53	
II$_{17}$I5	1.71	
II$_{17}$I6	1.66	
II$_{18}$I	1.36	1.632
II$_{182}$	1.30	
II$_{183}$	1.14	
II$_{184}$	1.33	
II$_{185}$	1.29	
II$_{187}$	1.10	
II$_{188}$	0.99	
II$_{189}$	1.06	
II$_{18}$I0	1.24	
II$_{18}$II	1.40	
II$_{18}$I2	1.36	
II$_{18}$I3	1.17	

TABLE VII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
IIssI4	1.30	
IIssI5	1.19	
IIssI6	1.11	
IIssI7	1.10	
IIss20	1.04	
IIss21	1.34	
		1.212
IIssI	1.27	
IIss2	1.24	
IIss3	1.40	
IIss4	1.37	
IIss5	1.39	
		1.334
IIesI	1.50	
IIes2	1.41	
IIes3	1.16	
IIes4	1.41	
IIes5	1.41	
IIes6	1.54	
IIes7	1.46	
IIes8	1.53	
IIes9	1.44	
IIesI0	1.43	
IIesII	1.56	
IIesI3	1.59	
IIesI4	1.57	
IIesI5	1.69	
IIesI7	1.44	
IIesI8	1.21	
		1.459
IImI	1.51	
IIm2	1.47	
IIm3	1.50	
IIm4	1.46	
IIm5	1.47	
IIm6	1.63	
IIm7	1.30	
IIm9	1.51	
IImI0	1.54	
IImII	1.33	
IImI2	1.46	
IImI3	1.41	
IImI4	1.53	
IImI5	1.47	
		1.471
IIemI	1.50	
IIem2	1.54	
IIem3	1.64	
IIem4	1.54	

TABLE VII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
IIe5	1.51	
IIe6	1.54	
IIe7	1.57	
IIe8	1.63	
IIe9	1.29	
IIe10	1.51	
		1.527
IIm1	1.76	
IIm2	1.80	
IIm3	1.54	
IIm4	1.74	
IIm5	1.49	
IIm6	1.71	
IIm7	1.56	
IIm8	1.70	
IIm9	1.57	
IIm10	1.43	
IIm11	1.41	
IIm12	1.71	
IIm13	1.61	
		1.617

Second Score of Generations; Temperature so Regulated that only Wingless forms Produced, a Six-day Interval Being Allowed for Growth and Reproduction of Each Generation.

For the second score of generations it was decided to make the conditions of the experiment more definite. Having learned the effect of temperature on the size of the individuals, it was decided to place them in a room where the temperature was less variable. This was done and temperature records were taken daily. During the whole of this part of the experiment no such variations in temperature were allowed as those which affected the first score of generations. By means of experiments elsewhere reported, I learned that under suboptimum growth conditions the winged forms would not appear. The optimum temperature for the wingless forms was found to be about 65° F. So well was the temperature controlled for the second twenty generations that not a single winged form appeared during the entire subexperiment. It was further decided that the same period of time should be allowed for growth and reproduction in each generation. The period allowed was 6 days.

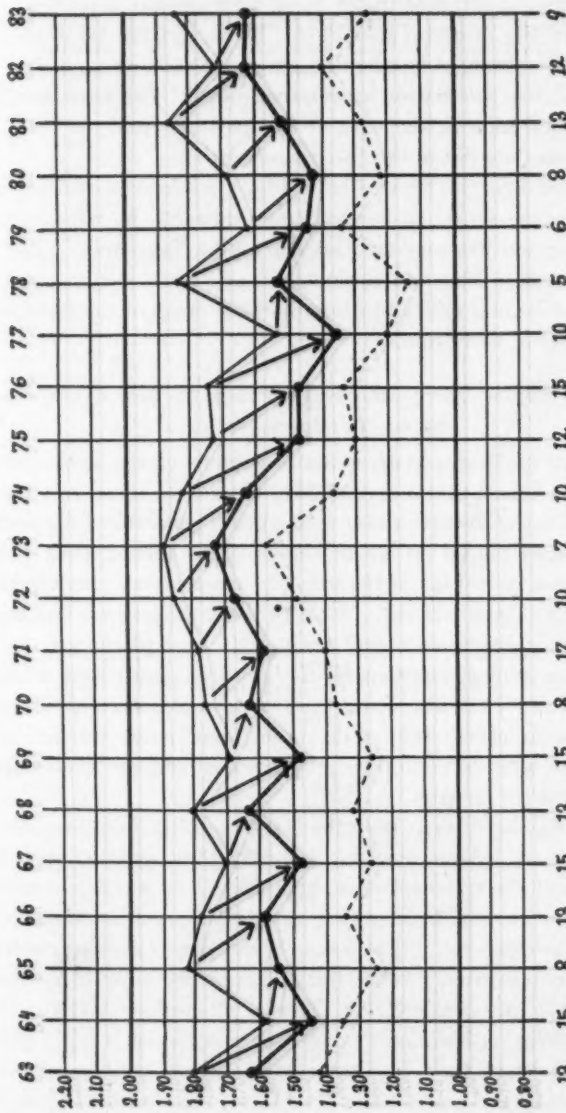


FIG. 8. Plotted results of selection in the second score of generations of isolation No. 11. Curves plotted on the same plan as in Fig. 7.

The results obtained under these more definite conditions are shown in Fig. 8 and Table VIII. By comparing these results with those obtained for the first 20 generations we note, first of all, much less fluctuation in fraternal means. We note that the extreme variants do not vary nearly as much from the mean of the line as they did in the first 20 generations.

We find that the heavy line in Fig. 8 joining the various fraternal means shows no general trend upwards, so we conclude that selection has produced no perceptible inherited change in body length. The last mean obtained, 1.611 mm., is just 6 thousandths of a millimeter less than the mean of the fraternity started with, 1.617 mm.

The Effect of the Use of Older Wheat Plants, the Last Four Generations of Isolation No. 11.

During the last 4 generations of subline 11 the aphids were reared on wheat plants several days older than those previously used. During the first and second score of generations the young aphids were placed on wheat shoots to be reared when these shoots were barely out of the soil. In almost every case the first leaf had not been put out. Most of these shoots were less than twenty-four hours old when the aphids were placed on them. In the last four generations all of the wheat plants were several days older. They were from 5 to 11 days old when the young aphids were added, and by the time these insects reached maturity and gave birth to their progeny most of these plants were two weeks old or older.

The offspring of 11_{831} were the first to be placed on these older wheat plants. After reaching maturity their lengths were obtained and the fraternal mean computed. It was found to be 1.343 mm., a big drop from that of the previous fraternity, which was 1.611 mm. The means of the three following generations were also greatly below this point and the mean of the line. The last mean obtained for these 4 generations and for the whole subline, or isolation 11 was 1.107 mm., which it is observed is greatly lower than any mean previously obtained and only 11 hundredths of a millimeter above the lowest variant obtained in all the first 40 generations where younger wheat shoots were used.

TABLE VIII.

CONTINUATION OF DATA FOR ISOLATION 11.—SECOND SCORE OF GENERATIONS.

Individual	Length in Millimeters	Fraternal Mean.
II$_{08}$2	1.50	
II$_{08}$3	1.51	
II$_{08}$4	1.36	
II$_{08}$5	1.50	
II$_{08}$6	1.47	
II$_{08}$7	1.41	
II$_{08}$8	1.41	
II$_{08}$9	1.39	
II$_{08}$10	1.47	
II$_{08}$11	1.40	
II$_{08}$12	1.44	
II$_{08}$13	1.39	
II$_{08}$14	1.44	
II$_{08}$15	1.59	
II$_{08}$16	1.43	
		1.447
II$_{09}$1	1.81	
II$_{09}$2	1.64	
II$_{09}$3	1.64	
II$_{09}$4	1.26	
II$_{09}$5	1.49	
II$_{09}$6	1.37	
II$_{09}$7	1.51	
II$_{09}$8	1.67	
		1.549
II$_{08}$1	1.39	
II$_{08}$3	1.70	
II$_{08}$4	1.47	
II$_{08}$5	1.77	
II$_{08}$6	1.77	
II$_{08}$7	1.43	
II$_{08}$8	1.69	
II$_{08}$9	1.66	
II$_{08}$10	1.49	
II$_{08}$11	1.46	
II$_{08}$12	1.79	
II$_{08}$13	1.74	
II$_{08}$14	1.34	
		1.592
II$_{07}$1	1.61	
II$_{07}$2	1.27	
II$_{07}$3	1.49	
II$_{07}$4	1.60	
II$_{07}$5	1.27	
II$_{07}$6	1.50	
II$_{07}$7	1.70	
II$_{07}$8	1.56	

TABLE VIII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
II$_{67}$9	1.51	
II$_{67}$10	1.29	
II$_{67}$11	1.60	
II$_{67}$12	1.64	
II$_{67}$13	1.38	
II$_{67}$14	1.46	
II$_{67}$15	1.44	
		1.487
II$_{68}$1	1.77	
II$_{68}$2	1.73	
II$_{68}$3	1.40	
II$_{68}$4	1.80	
II$_{68}$5	1.64	
II$_{68}$6	1.70	
II$_{68}$7	1.50	
II$_{68}$8	1.31	
II$_{68}$9	1.76	
II$_{68}$10	1.36	
II$_{68}$11	1.77	
II$_{68}$12	1.60	
II$_{68}$13	1.66	
		1.615
II$_{69}$1	1.44	
II$_{69}$2	1.69	
II$_{69}$3	1.36	
II$_{69}$4	1.50	
II$_{69}$5	1.60	
II$_{69}$6	1.53	
II$_{69}$7	1.41	
II$_{69}$8	1.53	
II$_{69}$9	1.67	
II$_{69}$10	1.40	
II$_{69}$11	1.61	
II$_{69}$12	1.47	
II$_{69}$13	1.54	
II$_{69}$14	1.31	
II$_{69}$16	1.26	
		1.488
II$_{70}$1	1.64	
II$_{70}$2	1.37	
II$_{70}$4	1.63	
II$_{70}$5	1.76	
II$_{70}$6	1.63	
II$_{70}$8	1.60	
II$_{70}$10	1.64	
II$_{70}$11	1.67	
		1.617
II$_{71}$1	1.44	
II$_{71}$2	1.50	

TABLE VIII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
II n3	1.57	
II n4	1.79	
II n5	1.59	
II n6	1.53	
II n7	1.66	
II n8	1.60	
II n9	1.66	
II n10	1.54	
II n12	1.66	
II n13	1.40	
II n14	1.64	
II n15	1.60	
II n16	1.69	
II n17	1.70	
II n18	1.49	
		1.592
II m1	1.71	
II m2	1.71	
II m3	1.51	
II m4	1.66	
II m5	1.66	
II m6	1.67	
II m7	1.69	
II m8	1.49	
II m9	1.86	
II m10	1.77	
		1.673
II n1	1.57	
II n2	1.81	
II n3	1.61	
II n4	1.89	
II n5	1.64	
II n6	1.67	
II n7	1.84	
		1.718
II n1	1.81	
II n4	1.64	
II n5	1.40	
II n6	1.49	
II n7	1.63	
II n8	1.79	
II n9	1.63	
II n10	1.37	
II n11	1.59	
II n12	1.79	
		1.614
II m1	1.50	
II m2	1.44	

TABLE VIII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
II713	1.54	
II714	1.41	
II715	1.39	
II716	1.34	
II717	1.54	
II718	1.46	
II7110	1.34	
II7111	1.69	
II7112	1.73	
II7113	1.30	
		1.473
II761	1.44	
II762	1.44	
II764	1.46	
II765	1.33	
II766	1.74	
II768	1.56	
II769	1.36	
II7610	1.47	
II7611	1.46	
II7612	1.60	
II7613	1.41	
II7614	1.33	
II7615	1.46	
II7616	1.54	
II7618	1.43	
		1.469
II771	1.53	
II772	1.36	
II773	1.44	
II774	1.39	
II775	1.20	
II776	1.24	
II777	1.39	
II779	1.34	
II7710	1.34	
II7711	1.49	
		1.372
II781	1.84	
II783	1.14	
II784	1.34	
II786	1.73	
II788	1.54	
		1.518
II791	1.34	
II792	1.51	
II793	1.61	
II794	1.43	

TABDE VIII. (continued).

Individual	Length in Millimeters	Fraternal Mean.
II795	1.53	
II796	1.36	1.463
II801	1.69	
II802	1.34	
II803	1.33	
II804	1.50	
II805	1.46	
II806	1.36	
II807	1.37	
II808	1.21	1.407
II811	1.36	
II812	1.57	
II813	1.37	
II814	1.63	
II815	1.43	
II816	1.86	
II817	1.59	
II818	1.50	
II819	1.46	
II8110	1.34	
II8111	1.50	
II8112	1.77	
II8113	1.27	1.511
II821	1.71	
II822	1.69	
II823	1.40	
II824	1.59	
II825	1.69	
II826	1.59	
II827	1.69	
II828	1.63	
II829	1.69	
II8211	1.43	
II8212	1.63	
II8213	1.60	1.612
II831	1.84	
II832	1.59	
II833	1.54	
II834	1.69	
II835	1.69	
II836	1.26	
II837	1.79	
II838	1.49	
II839	1.61	1.611

The results obtained for these last 4 generations of subline 11 are further illustrated in Fig. 9 and Table IX. Older plants, therefore, cause a remarkable diminution in the size of *Aphis avenæ* individuals. Perhaps it is because these aphids are not able to extract as much food from the harder tissues of older plants, but it may be due to a change in the nature of the juices sucked from the food plant, or due to both of these causes.

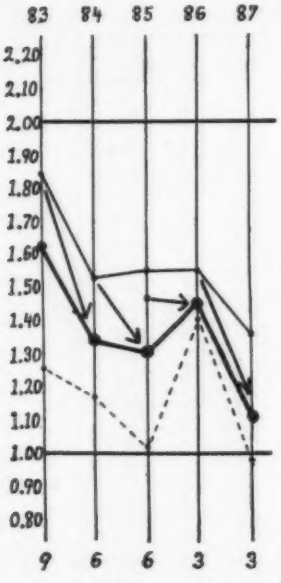


FIG. 9. Plotted results of selection in the last four generations of isolation No. 11. Curves plotted on the same plan as in Figs. 7 and 8.

The Effects of Selection for 44 Generations on a Fluctuating Variation.

The results of selection in the whole subline 11 are shown graphically in Fig. 10. Here we have plotted the exact lengths of each individual selected, scale ×50, and also the lengths representing each fraternal mean, plotted to the same scale. The barred vertical columns represent the lengths of the individuals selected, and the solid vertical columns represent the lengths expressed by the fraternal means. These two kinds of columns are arranged in the diagram Fig. 10 so that the two belonging to the same generation come in pairs, the number of the generation being given at the top of the dotted vertical guide line. The curved arrows, each of which passes from the top of the column representing the length of the individual selected, to the top of the column representing the length of the mean of its offspring in the next generation, shows the amount of regression, or digression, as the case may be, for the fraternal mean in question.

If we examine this figure we find that with but a single exception there is a dropping of the fraternal mean downward. The

mean of the fraternity for the sixtieth generation is found to represent a length slightly greater than that of the parent of this fraternity.

Is there any evidence of inheritable effects of selection in this long pedigree of 44 generations? Our only conclusion must be in the negative. There is not the least evidence of a general trend upward in the fraternal means as we advance generation

TABLE IX.

CONTINUATION OF DATA FOR ISOLATION 11.—LAST FOUR GENERATIONS.

Individual	Length in Millimeters	Fraternal Mean.
II$_{41}$1	1.47	
II$_{41}$5	1.17	
II$_{41}$6	1.51	
II$_{41}$9	1.24	
II$_{41}$10	1.27	
II$_{41}$11	1.40	
		1.343
II$_{42}$2	1.54	
II$_{42}$3	1.36	
II$_{42}$4	1.47	
II$_{42}$5	1.10	
II$_{42}$6	1.36	
II$_{42}$7	1.01	
		1.307
II$_{42}$1b	1.54	
II$_{42}$2b	1.39	
II$_{42}$3b	1.40	
		1.443
II$_{37}$5	0.99	
II$_{37}$6	0.97	
II$_{37}$7	1.36	
		1.107

after generation in this isolated subline. The fluctuations are many and at times great, but the series continues on in the same general way. These fluctuations have been partially analyzed, and in those cases where extremely low or high variatons occurred they were found to depend upon environmental conditions (temperature and food supply chiefly). If there is a cumulative effect in selecting extreme variants of each fraternity for a large series of generations, the results obtained in this long series of my pure line of *Aphis avenæ* Fab. fail to show it.

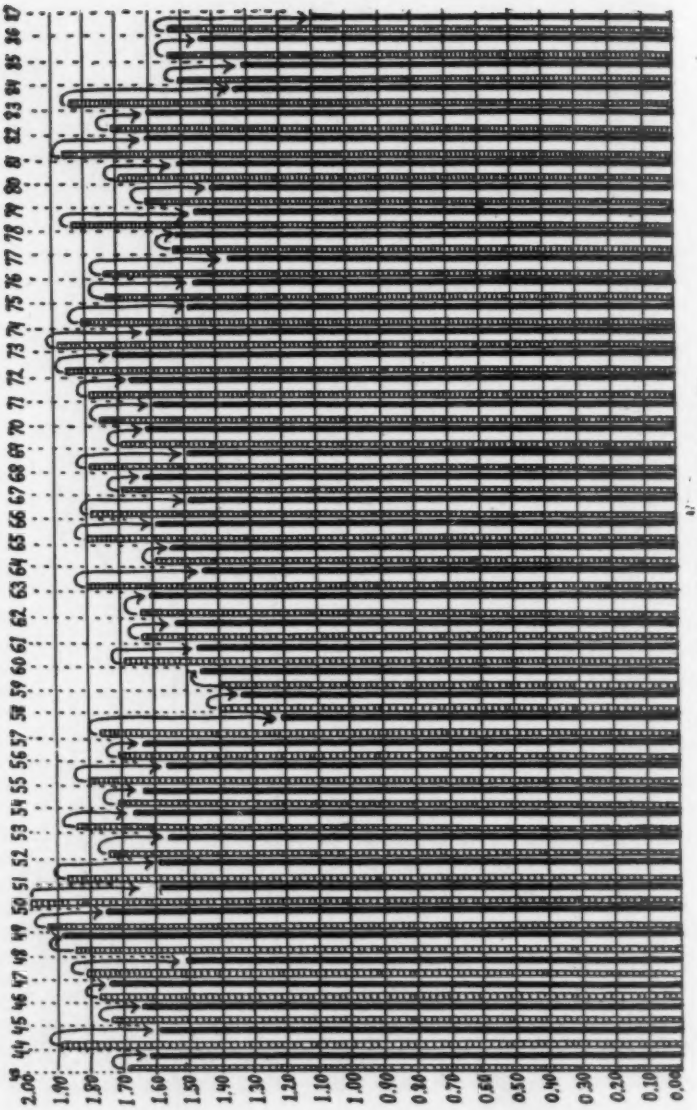


FIG. 10. For explanation see text, page 88.

The Effects of Continued Parthenogenetic Reproduction on the Virility and Metabolism of a Strain.

Whether or not long-continued asexual reproduction will affect the virility of a strain of animals has long been a debated question. The time was when zoölogists in general held that conjugation or some form of amphimixis was absolutely necessary in order to prevent the eventual dying out of a strain of animals that had long been reproducing asexually. The more recent work of Woodruff,[1] however, has shown us that in the case of *Paramœcium*, at least, conjugation is not necessary in order to rejuvenate a race. The question that next suggests itself is; can insects reproduce indefinitely by means of parthenogenesis? Probably the prevailing opinion among entomologists has been that parthenogenesis could not continue indefinitely in any species of the insects. Certain it is that in all those well-studied species where parthenogenesis is known to occur males have been found to exist. In fact, we find that frequently the parthenogenetic offspring are all males, and are produced thus according to definite law. Further, in the case of some insects, as aphids, males are produced according to the season. On the other hand, in the case of some insects that have not been sufficiently studied, males are unknown. In some of the species of *Thysanoptera* females have been found by hundreds, yet no males are known. In the case of our common pear-slug saw-fly, *Eriocampoides limacina* Retzius, I have counted hundreds, and reared scores of individuals without ever seeing a male, yet males are supposed to exist. Males must be extremely rare in the case of our common oyster-shell scale, *Lepidosaphes ulmi* Linn. I have reared scores and examined thousands of individuals without ever finding a male; however, the male has been described.

Since taking up this work with *Aphis avenæ* Fab., I have observed this species to pass the entire winter on the *Pacific coast* in the agamic form, and, as a matter of fact, have never observed the sexual form in that region of the country, although it probably exists there to a limited extent. We have several experiments on record of aphids having been reared partheno-

[1] Woodruff, L. L. For a review of the work of Woodruff see, Middleton, A. R., 1913, "Work on Genetic Problems in Protozoa at Yale," *Amer. Nat.*, Vol. XLVII., pp. 434–439.

genetically for more than half a hundred generations, but in such cases, I believe, no checks have been made to see if deterioration of the stock was taking place.

After breeding *Aphis avenæ* Fab. for 73 generations parthenogenetically, I found the species breeding at Ames, Ia., on oats and barley that was being raised for experimental purposes. I decided to start a check strain with these Iowa forms to see if

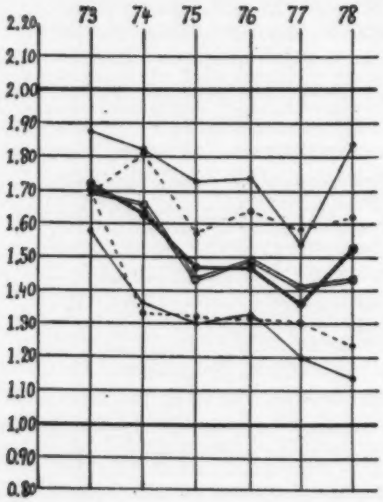


FIG. 11. Plot showing results obtained by running the Iowa strain of *Aphis avenæ* Fab. as a check on the Oregon strain to show the effects of long-continued parthenogenetic reproduction. The solid lines are for the fluctuations in the Oregon strain, the others for the Iowa strain. Plan of curves the same as in Figs 7, 8, and 9.

the continued parthenogenetic reproduction had reduced the virility of the experimental line obtained from a wild agamic female in Oregon.

A careful comparison of the Iowa forms with those of the seventy-third generation in my pure line showed no structural differences. They were somewhat different in coloration, being a deep, dark green, while those being used in the experiment were a light yellowish green. The cinnamon areas about the bases of the cornicles were well marked, but apparently were not so

extensive as those in the seventy-third generation of the experimental pure line.

One individual was isolated from those obtained in Iowa, and was started in the production of a check strain when the individual of the II_{73} generation was selected for carrying on the experimental strain obtained from Oregon. The means obtained, and the lengths of the extreme variants of each fraternity are plotted in Fig. 11. The data for the Iowa check strain are given in tabular form in Table X. What results do we find when comparison is made between these two strains?

1. *In Regard to Size.*—By observing the plot showing the extremes and the mean of each of the fraternities we see that they very nearly coincide. The parent individual of the Iowa strain had a length very near that of the mean of the II_{73} fraternity, which was the fraternity I was rearing at the time I obtained this stem parent. Of the five means obtained for the five fraternities of the Iowa strain, three were slightly above and two slightly below the means of the corresponding fraternities of the Oregon strain.

If we compare the average mean obtained from the fraternal means of the 5 check generations of the two strains, we have:

Average for the fraternal means: Oregon strain = 1.489 mm.

Average for the fraternal means: Iowa strain = 1.479 mm.

The difference between these two averages (one hundredth of a millimeter) is an insignificant difference when we consider the great range in variation found among individuals born of the same parent. We find, therefore, that as far as size is concerned there has been no dwarfing or indication of lack of vigor in growth due to the long continued parthenogenetic reproduction in the Oregon strain (the main pure line of *Aphis avenæ* Fab.).

2. *In Regard to Color.*—When first found the Iowa strain of *Aphis avenæ* showed a much deeper coloration than those individuals of the Oregon strain that had been bred for 73 generations in confinement. The individuals of the Iowa strain, however, lost these deeper colors immediately after being reared under the same conditions as those of the Oregon strain, and were undistinguishable from individuals of the latter strain. The fact that the individuals of the Iowa strain were more deeply

TABLE X.

DATA FOR IOWA CHECK STRAIN.

Individual	Length in Millimeters	Fraternal Mean.
I_{21}	1.57	
I_{22}	1.81	
I_{23}	1.73	
I_{24}	1.34	
I_{25}	1.77	
I_{26}	1.66	
I_{27}	1.59	
I_{29}	1.59	
I_{210}	1.57	
		1.625
I_{31}	1.41	
I_{32}	1.40	
I_{33}	1.41	
I_{34}	1.57	
I_{35}	1.49	
I_{36}	1.31	
		1.432
I_{41}	1.50	
I_{42}	1.49	
I_{43}	1.44	
I_{47}	1.31	
I_{48}	1.64	
		1.476
I_{51}	1.59	
I_{52}	1.36	
I_{53}	1.59	
I_{54}	1.33	
I_{55}	1.33	
I_{56}	1.40	
I_{57}	1.41	
I_{59}	1.34	
I_{510}	1.44	
I_{511}	1.57	
I_{512}	1.30	
		1.424
I_{61}	1.46	
I_{62}	1.24	
I_{63}	1.53	
I_{64}	1.61	
I_{65}	1.34	
I_{66}	1.60	
I_{67}	1.33	
I_{68}	1.40	
I_{69}	1.41	
I_{610}	1.49	
		1.441

colored when first found was probably due to the fact that they had been feeding on the leaves of the oat plants which contained much more chlorophyll than the tender shoots of wheat, upon which the individuals of the main pure line were being fed. At any rate the check experiment showed that they were not dependent upon a greater vigor or virility.

3. *Fecundity.*—From the data here obtained it is not easy to compare satisfactorily the fecundity of the individuals of the two strains. Six days was always the minimum time allowed for the development and production of young of each individual. Using this six-day life cycle schedule, however, we find considerable difference between the total number of individuals obtained from the five parents concerned. In the case of the Oregon strain 55 young were produced. In the case of the Iowa strain kept under identical conditions as a check, only 46 individuals were obtained. This gives for the average size of the fraternities of the Oregon strain 9.93 individuals, and for the fraternities of the Iowa strain an average of 7.44 individuals. We find, however, that one other factor enters into the results to such an extent as to make these findings of little value for comparing the relative fecundity of the two strains. This factor is the length of the period of development.

4. *Length of Period of Development.*—Of the 55 individuals obtained in the Oregon strain during the 5 test generations, only 2 failed to reach the paternity age in the 6 day period allowed. In the 46 individuals obtained at the same time in the 5 generations of the Iowa strain 8 individuals failed to reach the age of paternity in the 6 days allowed.

We find, then, that the Iowa strain developed much more slowly under the same conditions than the Oregon strain. I believe that the Oregon strain had changed its developmental period, since it was started about 2 years previously

What then was the effect of continued parthenogenetic reproduction for 73 generations in *Aphis avenæ* upon the virility of the strain? We find that by the means of comparison just reported no evidence of racial deterioration or loss of virility in any respect. We do find, however, strong evidences of adaptation in regard to the shortening of the growth period. Yet in

this regard we must remember that the Iowa strain with which this long-pedigreed Oregon strain was compared may have differed from the latter genetically. Our conclusions can not be final in regard to this point.

The Optimum Temperature for the Production of Wingless Agamic Forms, and the Relation of Temperature to Dimorphism.

During the early stages of the propagation of isolation 11 it appeared best to determine with stock obtained from discards of this subline the optimum temperature for the production of wingless agamic forms, and to see if it was possible so to regulate the temperature that only wingless forms would appear. Experience had shown that the growth of certain generations had evidently been retarded by unusual temperatures, and to obtain an optimum for growth would hasten the breeding very materially, and at the same time furnish more stable conditions for the experiment. The appearance of occasional winged forms during the first score of generations caused a complete loss, as far as number of individuals used in the experiment was concerned, as none of these was measured. If these winged individuals could be prevented from appearing, and the line made completely monomorphic, a great gain would be accomplished.

Accordingly mothers were placed in a constant temperature cell, and the young were allowed to be born and reared at various constant temperatures. The cell, which admitted light, was so regulated by an electric thermo-regulator that it seldom varied either way more than 0.2° F. The temperatures at which these various individuals were born and reared were as follows: 60° F., 70° F., 80° F., and 90° F. At 60° F. all the individuals reared produced winged forms. At 70° F. only 15.1 per cent. were winged; at 80° F., 69.6 per cent. were winged, and at 90° F. no individual lived to reach the last nymphal stage.

From these results I judged that at 65° F., or slightly above that, probably only wingless forms would be produced; certainly only very few winged forms should appear. These predictions were correct, for in the next 20 generations I so regulated the temperature for isolation 11 that not a single winged individual

appeared. Temperature changes alone, therefore, are capable of affecting dimorphism. Upon the tender shoots of wheat I produced either winged or wingless forms at will. It was a surprise to me to find that both extremes of temperature caused the appearance of the winged forms, and suppressed apparently the appearance of wingless forms.

How did these different temperatures affect the growth period of the wingless individuals? At 60° F., no wingless forms were produced; at 70° F. the average period of growth was 6 days;

TABLE XI.

DATA SHOWING EFFECTS OF VARIOUS CONSTANT TEMPERATURES ON APHIS AVENÆ FAB.

	At 60° F.	At 70° F.	At 80° F.	At 90° F.
Average daily birth rate per individual.	2.2	3.7	6.1	0.25
Length of developmental period for wingless form	——	6 days	7 days	12+days
Length of developmental period for winged form	14½ days	9½ days	7½ days	——
Mean length of adult wingless forms	——	1.155 mm.	1.246 mm.	——
No. of individuals used	44	37	61	5
Percentage of individuals winged	100.	15.1	69.6	——
No. of individuals reaching maturity	12	11	46	0
Percentage of mortality	72.7	70.3	24.6	100.

at 80° F., 7 days; at 90° F., 12+ days. It appears from this that the optimum temperature for the growth of wingless individuals is very nearly the same as the optimum temperature for the production of the same kind of individuals. It may be these two optima are the same.

The results of these experiments with constant temperatures, which gave additional data soon to be considered, are given in tabular form in Table XI.

THE EFFECT OF TEMPERATURE ON GROWTH, SIZE, REPRODUCTION, AND MORTALITY.

While starting the constant temperature experiment primarily to determine the optimum for the production of wingless agamic forms, it appeared profitable to observe also the effects of different constant temperatures on growth, size, reproduction and mortality.

Sources of Error.—Although there were important changes in humidity during these constant temperature experiments, yet, as has already been stated, Headlee has shown that changes of humidity from 37 per cent. to saturation are without effect upon the metabolism of plant lice. As for light changes, it is probable that metabolism was to some extent affected by these, but experience with other insects has shown that unless these are quite marked (*e. g.*, bright sunlight or total darkness) the effects are not great. There was no change in the food, unless perhaps the changes in temperature themselves changed somewhat the nature of the food derived from the wheat shoots.

The results obtained showing the effects of the four constant temperatures employed; 60° F., 70° F., 80° F., and 90° F., are given in tabular form in Table XI.

1. *As to Growth.*—At 60° F. it took the winged forms 14½ days to reach maturity (this being the average for all individuals reared). No wingless forms were produced at this temperature, so that we have no data upon the growth period for this form at this period. At 70° F., the wingless forms reached maturity in 6 days, the winged in 9½ days. At 80° F., the wingless forms reached maturity in 7 days, the winged in 7½ days. At 90° F., no nymphs of the winged forms had reached maturity in 12 days. No nymphs of wingless forms were observed at this temperature, and all the individuals used died before reaching maturity. The optimum temperature for growth, therefore, must be different for the two forms. For the wingless forms it probably is a little above 65° F. as before stated, but in the case of the winged forms is somewhere near 80° F.

2. *As to Size.*—Only two records for the size of adult wingless forms were obtained. The mean for those reared at 70° F. was 1.155 mm., and for those at 80° F., 1.246 mm. Both these means are below the mean of the pure line stock from which the mothers were taken, and far below the mean! Can it be that constant temperatures, no matter at what degree, have a dwarfing effect on plant lice? Does the changing of temperature stimulate growth and metabolism in general? These are interesting problems for further investigation. The results obtained in this experiment appear to show that such dwarfing actually does take

place at ordinary temperatures if the temperature is kept constant.

3. *As to Rate of Reproduction*—At 60° F. the average rate of reproduction was 2.2 births per day per individual. At 70° F. the rate was 3.7 per day per individual. At 80° F. the rate was 6.1 and at 90° F., 0.25. Therefore, we conclude that temperature affects the rate of reproduction in a most fundamental way; that about 80° F. is the optimum temperature for reproduction; and, that the optimum for reproduction is quite different from the optimum for growth, at least as far as the wingless forms are concerned.

4. *Effect on Mortality.*—The mortality percentages for the different constant temperatures were: 60° F., 72.7; 70° F., 70.3; 80° F., 24.6; 90° F., 100. Obviously, the extremes of temperature are quite disastrous to *Aphis avenæ*. At 60° F. we find that almost 3 out of 4 die before reaching maturity. At a constant temperature of 90° F. not a single individual reached maturity. This observation coincides well with observations in general of specialists in the Aphididæ, who report the disastrous effects of hot summer weather on our injurious species. The optimum for the least mortality appears to near 80° F., but probably it is slightly below this point.

The Effect of Fasting, During the Growth Period, on the Size of Adults.

The abundance of food during the growth period of an insect is known frequently to affect the size of the adults produced. It occurred to me that probably fasting periods of different lengths during the growth of individuals of *Aphis avenæ* Fab. might affect the size of the adults. In order to test this point, 33 young nymphs, born between 6:00 and 12:00 A.M. (average time of birth 9:00 A.M.), were placed in different lots in four different breeding cages, but under similar conditions, and were deprived of food for different periods. The individuals of all lots were allowed to feed for 24 hrs., then they were deprived of food for the following periods: Lot 1, 4 hrs.; lot 2, 8 hrs.; lots 3 and 4, 24 hrs. At the end of these fasting periods it was found that the individuals of lots 3 and 4 were dead. The others endured their

shorter fast and were placed back on their succulent wheat shoots in order that they might complete their development. Of those in lot 1, 2 individuals reached maturity, and of those in lot 2, 4 individuals reached maturity. The average time required for the individuals of these two lots to reach maturity was 5 days in both cases. Quite normal. They were all wingless forms, and gave the following measurements for length: Lot 1, 1.00 mm. and 0.89 mm.; lot 2, 0.96 mm., 0.99 mm., 0.97 mm., and 1.00 mm. The mean lengths for the two lots were: Lot 1, 0.945 mm.; lot 2, 0.980 mm. Both are much bleow the mean for the line.

TABLE XII.

DATA FOR EXPERIMENT ON EFFECT OF SINGLE FASTING PERIODS ON SIZE OF ADULTS.

Lot No.	No. Individuals in Lot.	Period in Which Birth Took Place.	Average for Date of Birth.	First Feeding Period.	Length of Fast.
1	4	6.00 to 12.00 A.M.	9.00 A.M.	24 hrs.	4 hrs.
2	7	6.00 to 12.00 A.M.	" "	" "	8 hrs.
3	6	6.00 to 12.00 A.M.	" "	" "	24 hrs.
4	10	6.00 to 12.00 A.M.	" "	" "	24 hrs.

TAELE XII.—*Continued.*

Lot No.	First Individual Adult.	Last Individual Adult.	Length of Life Period, Average.	Winged or Wingless.	Mean Length for Lot, in Mm.
1	Jan. 8, 9 A.M.	Ja. 8, 9 A.M.	5 days	All wingless	0.945
2	" "	" "	"	" "	0.980
3	All dead				
4	All dead				

From this we conclude that single fasts during the development of *Aphis avenæ* Fab. do not necesarily change the length of the period of development, but greatly reduce the size of the adult individuals produced. Data for this fasting experiment are given in tabular form in Table XII. It will be noticed that all the individuals produced in this experiment were wingless forms.

THE RANGE AND NATURE OF VARIATION IN A PARTHENOGENETIC PURE LINE.

Variation in a parthenogenetic pure line presents all the essential features that it does in nature in general. Probably the one thing that impresses one more than anything else in

regard to variations in a parthenogenetic pure line is the great predominance and great range of fluctuating, or individual, variations; including under this head the variations in parts of the body as well as variations affecting the whole of it. These are the omnipresent, the conspicuous, and, I am tempted to state, the characteristic variations in a parthenogenetic pure line. It is true that we occasionally find an abrupt, or spontaneous variation, but such variations are rare, and as a rule are in the nature of omissions of parts or the arrest of the development of the whole or a part of the individual. Fluctuating variability has been shown by several workers to be greater where amphimixis does not take place. Walton[1] has shown this conclusively for *Spirogyra*. Hence, we may assume in the pure line of *Aphis avenæ* here considered that the absence of amphimixis is a fundamental reason for the great range in fluctuating variability.

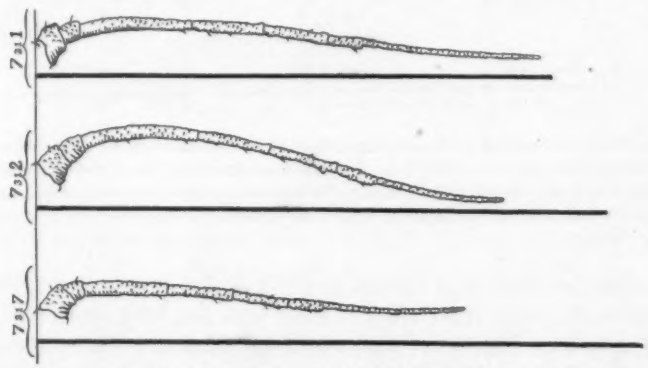


FIG. 12. Camera lucida drawings, all of the same magnification, showing extremes of fluctuating variation in the lengths of the right antennæ of three individuals (7_{31}, 7_{32}, and 7_{37}) of the 7_{31} fraternity. The heavy black line in each case represents the body length, same magnification, of the individual whose antenna is drawn by the side of it.

The range of fluctuating variability is frequently so great among the individuals of a single fraternity as to be quite noticeable to the eye without measurements being made. In Fig. 12 we have camera lucida drawings, all of the same magnification of the right antennæ of three individuals of the 7_{31} fraternity. Also

[1] Walton, L. B., 1915, "Variability and Amphimixis," *Amer. Nat.*, Vol. XLIX., pp. 649–687.

by the side of each antennal drawing is a heavy line representing the length of the individual possessing the antenna, also magnified to the same degree as the antenna itself. In the case of individual $7_{31}1$ the antenna is practically of the same length as the body. In the case of $7_{31}7$ the antenna is considerably less than two thirds as long as the body. All intergrades were found between these two extremes, showing that these variations are only fluctuations.

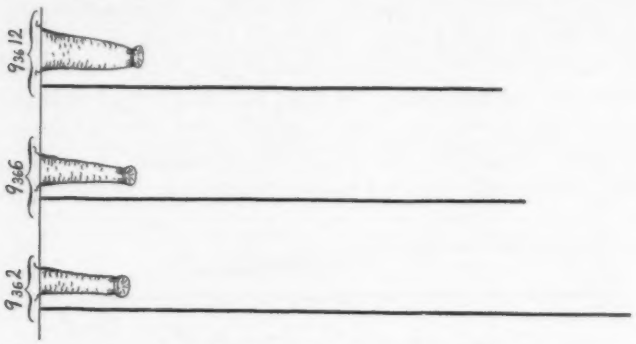


FIG. 13. Camera lucida drawings, all of the same magnification, showing extremes of fluctuating variation in the lengths of cornicles from three individuals ($9_{36}12$, $9_{36}6$, $9_{36}2$) of the 9_{36} fraternity. The heavy black line in each case represents the body length, same magnification, of the individual whose cornicle is drawn by the side of it.

Fig. 13 shows us a similar variation in the length of the cornicles in comparison with the length of the body in a single fraternity. The cornicles of individual $9_{36}12$ (the left of which is shown at top of Fig. 13) are 25.5 per cent. of the total body length of the same individual. The cornicles of individual $9_{36}2$ (one of which is represented at the bottom of Fig. 13) are only 17.3 per cent. of the body length of this individual.

The variation in the relative size of contiguous parts is shown in Fig. 14, where we have represented camera lucida drawings, all of the same magnification, of the third and fourth segments of the antennæ of three different individuals of the F_9 fraternity. Here the large segments are the third and the small ones the fourth. Observe that there is almost no variation in the length

[1] The letter *F* is here used to denote the subline or isolation, and refers to isolation No. 3 (only one isolation was running during the ninth generation).

of the fourth segment, but a great variation in the length of the third. In $F_9$16 the third segment is not more than a fourth longer than the fourth segment; in $F_9$15 the third segment is almost twice as long as the fourth one.

Variation in size is probably as noticeable as any other of the fluctuating variations. In Fig. 15 we have drawings of the same magnification of two individuals obtained in isolation 11 of

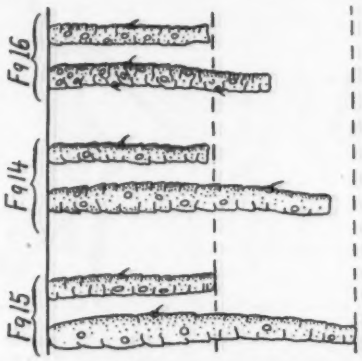


FIG. 14. Camera lucida drawings, all of the same magnification, showing the extremes of fluctuating variation in the length of the third as compared with the fourth segment of the antenna of three individuals of a single fraternity (F_9). The third (long) and fourth (short) segments of an antenna of a single individual are drawn together to same scale.

the pure line of *Aphis avenæ*. The larger figure is of individual 11_{501}, length 1.99 mm.; the smaller one of 11_{558}, length 0.99 mm. In other words one individual is slightly more than twice as long as the other, and from this we should judge it to be about 8 times as heavy (the two not being weighed). These are the extremes obtained in 459 individuals, representing all those obtained during the first 40 generations of isolation 11.

Not only do we find fluctuations in regard to size in the individual and its parts, but in shape also. In Fig. 16 we have drawings of the right cornicles of three individuals of the 4_{27} fraternity. What a variation in shape! In the case of the right cornicle of 4_{271} we find the base almost twice as broad as the distal end. In the right cornicle of 4_{274} the base is no wider than the distal end. This latter cornicle is strongly incrassate at its tip,

while the former is not at all incrassate. Yet frequently the incrassate nature of the cornicles has been used as a generic character!

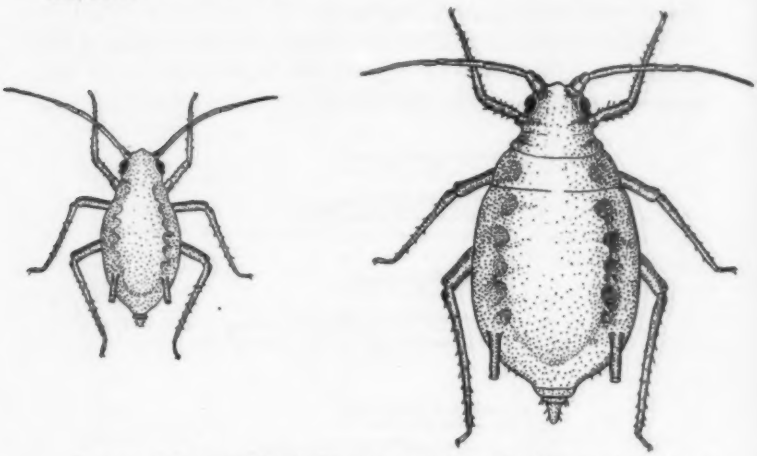


FIG. 15. Camera lucida drawings of same magnification showing extreme fluctuations in size of two individuals of isolation No. 11. The larger individual is 11_{401}; the smaller 11_{448}.

Do these fluctuating variations group themselves about a common mean as such variations normally do? Apparently

FIG. 16. Camera lucida drawings, all of the same magnification, showing the extremes of fluctuating variation in the shape of the cornicles of three individuals ($4_{17}3$, $4_{17}1$, and $4_{17}11$) of the same fraternity.

yes. In Fig. 17, I have plotted the frequency polygon for the character of body length for the 477 individuals obtained in

isolation 11. Notwithstanding the selection made in this subline we find the typical unimodal frequency polygon (shown by the heavy unbrokem line) representing the normal variations of a fluctuating character.

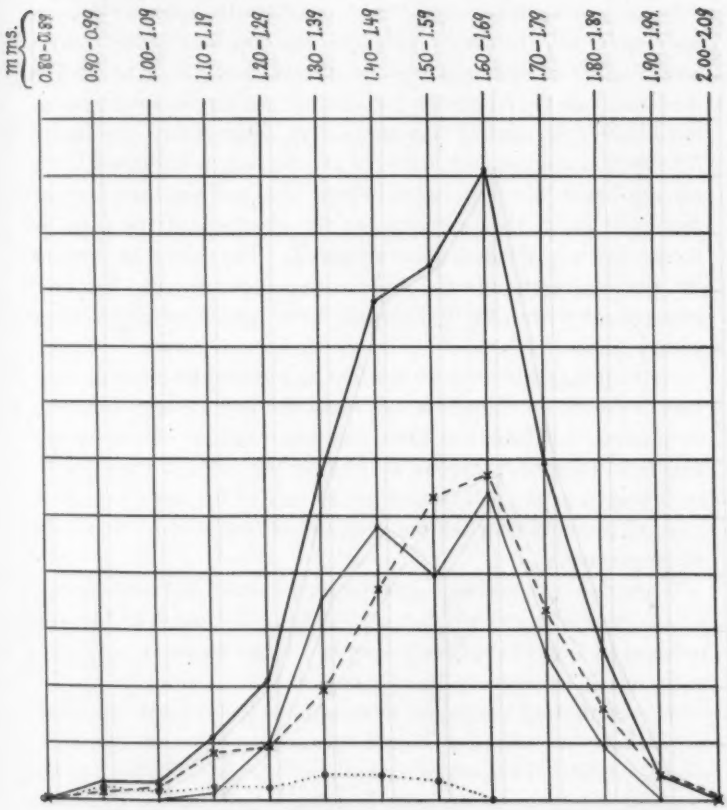


FIG. 17. Frequency polygon to show variations in body length for the 477 individuals obtained in isolation No. 11. The polygon is broken up into three components representing the frequency polygons for the first score, second score, and last four generations of this isolation. For further explanation see text.

The causes for the variation in fluctuations have not been well understood in most instances. De Vries maintains that in plants they depend probably exclusively upon nutrition. I find that

in plant lice temperature as well as nutrition is the cause of fluctuations in body length. The effect of temperature on the variations in body length is well shown in Fig. 17. The large frequency polygon represented by the heavy broken line at the top can be analyzed into three components representing respectively the frequency polygons for the first score, second score, and last four generations of isolation 11. The broken line describes the frequency polygon for the first score of generations where no control was made over temperature conditions. The light unbroken line describes the frequency polygon for the second score of generations where the temperature was so regulated about the optimum for the development of wingless forms that only wingless forms appeared. The dotted line toward the bottom represents the frequency polygon for the last four generations where the individuals were reared on much older wheat plants.

In studying the curves for the first and second scores of generations we note no change of the mean for body length which in both cases is a little less than 1.65 mm., but by observing the bases of these two polygons we find the extremes of variation in body length to be much greater in the case of the one for the first score of generations when the extremes of temperature were also much greater.

When a change was made in the nature of the food (temperature being left the same) we find a shifting of the mean (see lower polygon in Fig. 17) for body length. It no longer is near 1.65 mm., but is between 1.35 and 1.45 mm.

But fluctuating variations were not the only kinds observed in the pure line of *Aphis avenæ*. Some abrupt variations were also observed. These abrupt, or discontinuous, variations were, however, very rare in the pure line of the grain aphis. I found only four that were quite noticeable. One of these was a dropping out of the branch of the sector of the third discoidal vein in the anterior wings of the winged form, another the dropping out of the sector itself; the third, an addition of one or more cross veins to the bifurcating branches of the sector of the third discoidal vein; the fourth, the complete arrestment of development of the individual during the third or fourth nymphal stages.

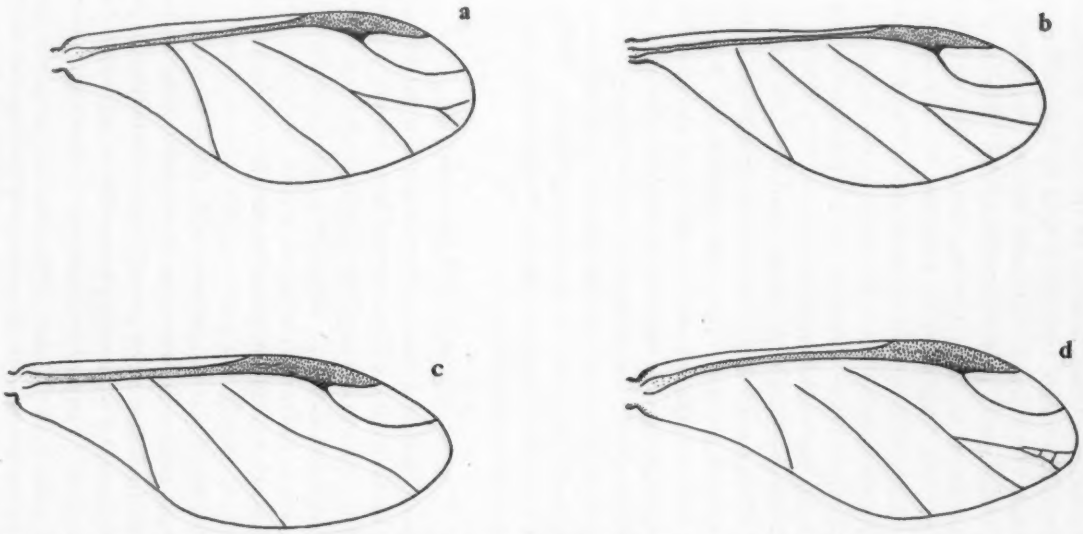


FIG. 18. Abrupt, or discontinuous variations in the pure line of *Aphis avenæ* Fab. *A*. Wing of winged form showing the normal branching of the third discoidal vein. *B*. Branch to sector of third discoidal vein missing. *C*. Sector itself wanting. *D*. Two cross veins between branches of sector of third discoidal vein.

I only studied the transmissibility of the latter abnormality. Where individuals stopped completely their development in any nymphal stage, they always produced normal young, and in every case these young grew to normal adults. The arresting of the development of these nymphs caused them to become pædogenetic. The pædogenesis, however, was never fixed. Several of these pædogenetic nymphs appeared. Some of them were nymphs of wingless form, and some of the winged form. The cause of the arrested development I did not learn, but it may have been due to low temperatures.

The variations mentioned in the third discoidal vein are shown in Fig. 18. I hope some time to test the transmissibility of these abnormal wing characters. The variation shown in *B* is quite common. The variations shown in *C* and *D*, on the other hand, are rare. In regard to the variations of this third discoidal vein, it should be mentioned that frequently the venation of the wing on one side of an individual will be different from the venation of the wing on the other side of the same individual. Yet there is a tendency for the anomaly to appear simultaneously on both wings of the same individual.

The Occurrence of Pædogenesis.[1]

The occurrence of pædogenesis (the reproduction parthenogenetically by immature instars) is a phenomenon known to be well established in the genus *Miastor* (Fam. Cecidomyiidæ), and occurs also in a few of the species of *Chironomus*. But pædogenesis is extremely rare in the animal kingdom, and as far as the writer can learn has not been recorded as occurring erratically in plant lice.

In the course of my experiments with *Aphis avenæ*, which covered about two and a half years, I observed on several occasions individuals that never passed beyond their third or possibly fourth nymphal instar, but nevertheless began and continued to reproduce in a normal manner. These pædogenetic

[1] The word pædogenesis is here used in a broad sense denoting parthenogenetic reproduction by any immature stage of an animal that passes through a metamorphosis. Nymphal pædogenesis does not have nearly as deep a significance as larval pædogenesis that is found in some insects which pass through a complete metamorphosis.

nymphs were of both forms (the wingless and winged), and in every instance their young gave only normal adults, and had so far as could be ascertained had normal reproductive powers. I shall here record some data obtained in regard to these pædogenetic nymphs.

One of the first paedogenetic individuals observed was $3_{18}5$. This nymph was of the winged form and never passed beyond the last nymphal stage. It was born from $3_{18}5$ along with 13 other young. It reached its final nymphal form at the time that two of its sisters reached their normal maturity. This pædogenetic nymph gave birth to 8 young the first day of reproduction, 4 the second, none on the third and fourth days, and was killed on

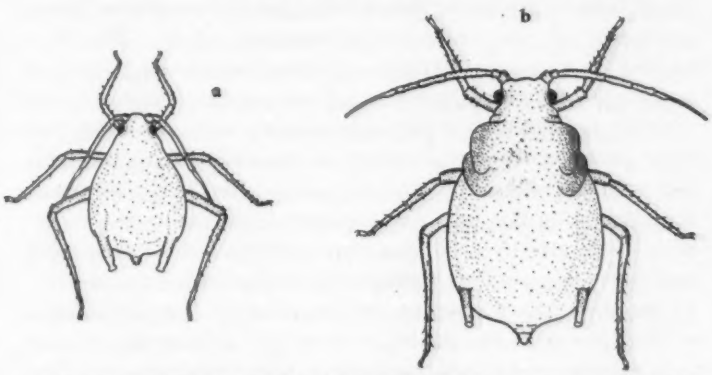


FIG. 19. Pædogenetic nymph of *Aphis avenæ* Fab. *A*. $3_{31}10$, a pædogenetic nymph of the wingless form. *B*. $11_{41}10$, a pædogenetic nymph of winged form.

the fifth day. Of the 12 offspring left, 3 reached maturity. They were normal wingless forms, and differed in no perceptible way from the many other wingless forms at hand. These three normal adults each gave many young, which, however, were not reared to maturity.

Another pædogenetic nymph to appear was $3_{24}10$ (see Fig. 19, *a*), which was a pædogenetic third nymph of the wingless form. It was one of the 12 offspring of $3_{23}1$, a normally winged adult. Of the 11 sisters of this pædogenetic form, 10 reached maturity, and all proved to be normal wingless forms. This pædogenetic nymph gave birth to 3 young before it was killed. These young, however, were discarded because of lack of time to care for them.

But the most noteworthy case of erratic pædogenesis was found, however, in the offspring of II_{485}, a normal wingless adult, that left 13 young. Of these 13 young, 9 reached the stage of maternity. Of these 9, 3 were pædogenetic, II_{491}, II_{492}, and II_{495}. These pædogenetic nymphs were all of the winged form, and reached their stage of reproduction along with the adults of the wingless form, which is much sooner than the time allowed for the development of the normal winged adult forms. The pædogenetic nymphs were all large, vigorous individuals, and reproduced normally. The individual II_{491} gave 11 young before she was killed. Of these young, 1 was reared to maturity, producing a normal wingless adult. Another, II_{492}, gave 14 young before being killed, and of these, 13 were reared to maturity, producing in every case normal wingless adults. The third individual, II_{495}, gave birth to 16 young before being killed, of which 4 were reared to maturity, all being normal wingless forms.

Other instances of erratic pædogenesis were met with, but these suffice to show the nature of their appearance, and the fact that this tendency toward pædogenesis is not inherited. Pædogenesis in this pure line appeared to be due to the arrestment of the growth of the immature individual, the development and functioning of the reproductive system being unhindered. It was probably induced by low temperature changes during a critical period in the development of the individual. At any rate adverse conditions must have been responsible for this arrested development.

Summary of Results.

In Regard to Heredity.

1. Six different fluctuating variations in a parthenogenetic pure line of *Aphis avenæ* Fab. were tested to see if there was any summation effect of selection on these variations. Selections were made for ten or more successive generations in the case of three of these characters; for forty-four successive generations in the case of one character; and were carried out in both plus and minus directions in the case of two characters. In all of these cases no summation effect was produced by selection.

2. The results of this work with *Aphis avenæ* Fab. are, we believe, sufficient to warrant the following generalization: Fluctuating variations in a parthenogenetic pure line of *Aphis avenæ* Fab., and presumably in all parthenogenetic pure lines, are in general not dependent upon germinal variations, and for this reason are not capable of increase or summation through the action of continued selection. Or to put it in another way: Fluctuating variability in a parthenogenetic pure line is devoid of one of its most important causes when exhibited in higher animals that reproduce sexually that is, germinal variability.

3. *Corollary.*—Some investigators, not considering the original limitations that were placed on the pure line theory, *i. e.*, that it applies only to forms that reproduce asexually or by self-fertilization, have attempted, and disastrously, to apply its principles to sexually reproducing animals. This has been unfortunate, and has added confusion to controversy, and has been wholly unnecessary. Castle's work with hooded rats[1] has shown conclusively that germinal variability is to be assigned as the cause, of a part at least, of phenotypical variability in these higher forms, thus exempting them from the scope of the pure line theory.

4. Selection from extreme variants within a pure line of *Aphis avenæ* Fab. is without effect upon the somatic characters of succeeding generations.

5. Long-continued selection from the extreme variant in each succeeding fraternity produces no more of a change in the mode of the variable than selection from individuals but slightly different from the mean of the line and for only a few generations. Neither produces any perceptible change.

6. The pure line theory applies to parthenogenetic arthropods as well as to forms that reproduce by budding, fission, or self-fertilization.

7. Selections from extreme variants for 44 consecutive generations, using a character that is well known to be inherited in higher animals that reproduce sexually, failed to shift in the least the mean for the line.

[1] See Castle, W. E., 1915, "Some Experiments in Mass Selection," *Amer. Nat.*, Vol. XLIX., pp. 713–726.

8. Where selections were made in opposite directions (plus and minus) in two isolations, each being used as a check against the other, it was found that the fluctuations were simultaneous in both isolations, and in the same directions, being thus independent of the effects of selection.

In Regard to General Biology.

9. Fluctuating, or individual variations, are of great magnitude, even in a single fraternity of a pure line of *Aphis avenæ* Fab., and are frequently different on the two sides of the same individual.

10. Abrupt, or discontinuous variations are very rare in a pure line of *Aphis avenæ* Fab., and the few that were observed were not proved to be inherited.

11. The variations in stature, or body length in *Aphis avenæ* Fab. are due largely to variations in temperature and food.

12. The optimum temperature for the production of wingless agamic forms in *Aphis avenæ* Fab. is about 65° F., and the percentage of winged forms produced increases in accord with the degree of divergence both above and below this optimum.

13. Only wingless agamic forms are produced at a mean average daily temperature of about 65° F.

14. A constant temperature of 90° F. is sufficient to prevent completely the development of *Aphis avenæ* Fab.

15. Long-continued parthenogenetic reproduction did not affect the size, color, or fecundity of the strain of *Aphis avenæ* Fab. used. The period of growth was somewhat shortened. This may have been an adaptation, or response of the strain to the artificial, suboptimum and almost constant conditions under which they were kept.

16. Pædogenesis occasionally occurs in *Aphis avenæ* Fab., both among the nymphs of the winged form and nymphs of the wingless form. It is due to the arrested development of the body in general, while the reproductive organs become completely functional. All offspring of these pædogenetic nymphs that grew to become adults were normal.

17. Fluctuating variations are the omnipresent, the conspicuous, and in fact the characteristic variations in a pure line of plant lice, and, as have before stated, they were not shown to be inherited.

THE TRANSFORMATION OF BRACHIONUS PALA INTO BRACHIONUS AMPHICEROS BY SODIUM SILICATE.

DAVID D. WHITNEY.

In certain species of rotifers there is considerable variation in the shape and form of the body of the female during the year. Powers has shown that in one species of *Asplanchna* there are three distinct phases through which a race may pass during one season. The females in each phase differing from those in either of the other two phases in both size and general form of the body. He and Mitchell also have shown that in this particular species these changes are somewhat corellated with the sexual stage of the females which is produced by changes in food. Sachse studied the variability of form in several other species and found that the greatest variability occurred at the periods of abundant food but he did not find that this variability was in any way correlated with the sexual stage. Lauterborn made observations upon *Anuraea cochlearis* and concluded that the variations of form were due to changes in temperature. Wesenberg-Lund made observations upon *Asplanchna priodonta* and concluded that the variations were due to differences in density of the water which varied according to the temperature.

Recently H. K. Harring, custodian of the Rotatoria in the United States National Museum, pointed out to me that *Branchionus amphiceros* was a variation of *Branchionus pala* and that it probably could be artificially produced from *Branchionus pala* at any time by changes in diets. Knowing, however, only one diet on which this species would thrive readily in the laboratory it was impossible to test this suggestion.

In considering the problem the self-evident fact was recalled that the young stages of all living organisms must be supplied with suitable materials out of which to build the various tissues of their bodies. If an animal has a lime or a siliceous skeleton it must be supplied with calcium or siliceous salts with which to

build its framework. In all rotifers there is some skeletogenous tissue and in most species the greater part of it forms the external covering. In some species this external skeleton is excessively delicate and flexible while in other species it is relatively thick and non-flexible. This external covering is usually considered as being composed of a chitinous material. In general the different species of rotifers are distinguished from each other by the different forms of this external covering. If one could furnish an abundance of material such as the animals use in making this covering it would seem that there might be an opportunity for obtaining variations in the form and size of the covering. Not knowing how to obtain chitin in a liquid form other materials were considered. Sodium silicate (water glass) was used first and was at once successful beyond all expectations.

Brachionus pala possesses two small posterior spines, one on each side of the base of the tail, whereas the variation *Brachionus amphiceros* not only possesses these two spines which are usually longer but also possesses in addition two large lateral posterior spines. These lateral spines vary somewhat in size in different individuals and in the same individual they are much larger in proportion to the size of the body in the young stage than in the adult stage.

During the spring and summer both forms are found but there is considerable irregularity in their appearance. In the spring *Brachionus pala* may be found almost exclusively, while later *Brachionus pala* and *Brachionus amphiceros* may occur in equal numbers and still later, from June to October, *Brachionus amphiceros* may form as high as 93 per cent. of the total collections of the two forms, as was found by Kofoid. The exact cause of the fluctuating appearance of these two forms in the same body of water is not known.

In the first series of experiments one drop, two drops, three drops, four drops, and five drops of sodium silicate were added respectively to five jars each containing 150 c.c. of beef bouillon culture medium. These jars were inoculated with pure cultures of the green flagellate, *Chlamydomonas*. As soon as there was a vigorous growth of the *Chlamydomonas* several dozen females of *Brachionus pala* were put into each jar. After a few days had

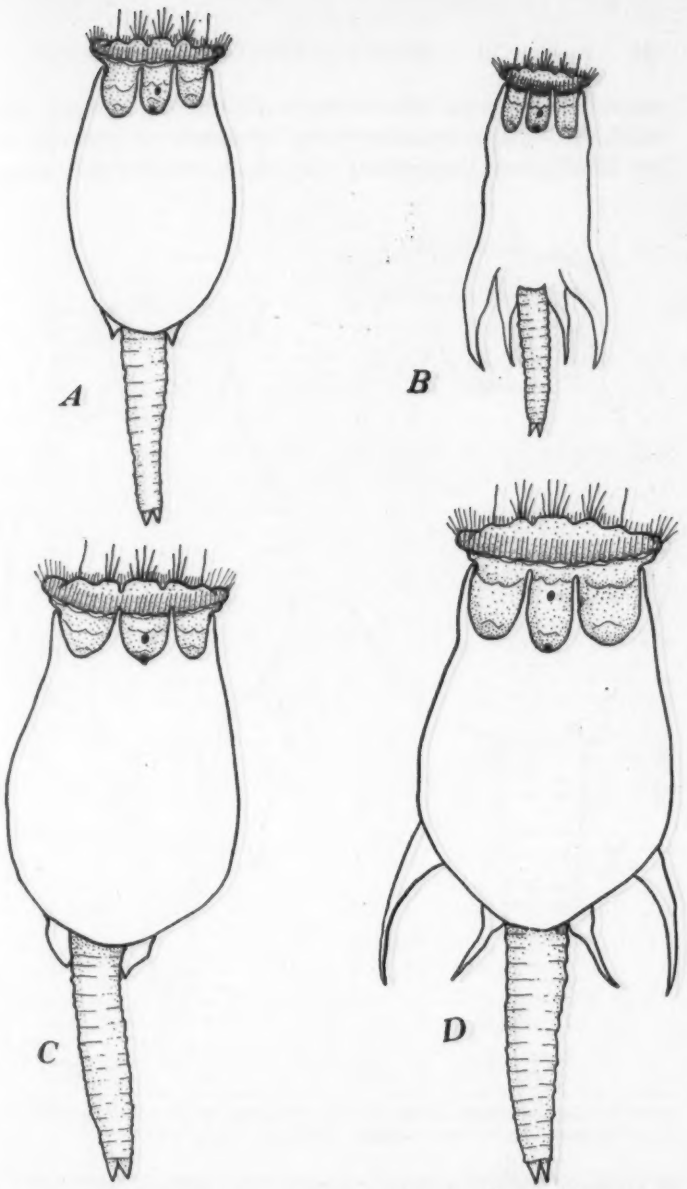


FIG. 1. *A*, young female just after emerging from a parthenogenetic egg which was produced in culture medium free from sodium silicate; *B*, young female just after emerging from a parthenogenetic egg which was produced in a culture medium containing sodium silicate; *C*, similar to *A* only older and larger; *D*, similar to *B* only older and larger.

elapsed observations were made on the rotifers in these jars which at this time contained many thousands. In the control jars free from sodium silicate only three females from nearly

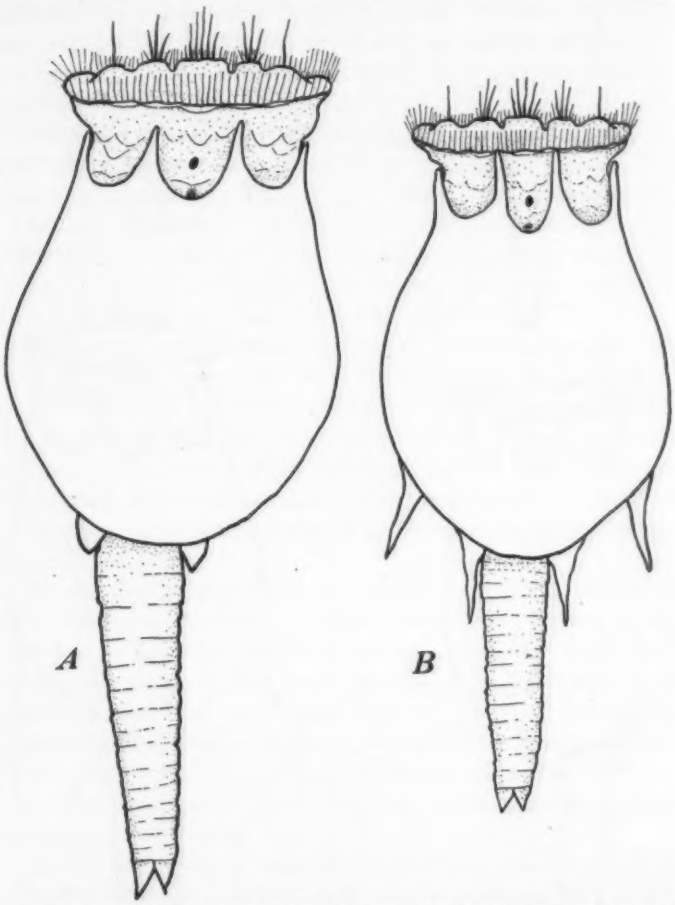


FIG. 2. *A*, adult female reared in culture medium free from sodium silicate; *B*, adult female reared in culture medium containing sodium silicate.

six thousand females possessed the posterior lateral spines while in the jars containing sodium silicate many of the females possessed the posterior lateral spines. In some of the jars containing

five drops of sodium silicate every female possessed these additional spines and in all the jars containing ten drops of sodium silicate every female also carried these spines. Not only did the

TABLE I.

SHOWING THE INFLUENCE OF SODIUM SILICATE IN THE PRODUCTION OF TWO ADDITIONAL POSTERIOR LATERAL SPINES IN THE FEMALES OF *Brachionus pala* THUS FORMING *Brachionus amphiceros*.

Experiments.	Observations, 1916.	Control Culture Medium Free from Sodium Silicate.			One Drop of Sodium Silicate in 150 c.c. Culture Medium.			Four Drops of Sodium Silicate in 150 c.c. Culture Medium.			Five Drops of Sodium Silicate in 150 c.c. Culture Medium.			Ten Drops of Sodium Silicate in 150 c.c. Culture Medium.		
		Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.	Females — Spines.	Females + Spines.	Females + Spines %.
1	Jan. 27....	998	2	.002	90	10	10	4	96	96	0	50	100	...	...	...
	Feb. 2.....	1,000	0	0	88	12	12	3	97	97	0	200	100	...	...	...
	Feb. 7.....	100	0	0	...	...	...	0	100	100	0	100	100	...	...	...
	Feb. 14....	99	1	1	...	...	...	0	100	100	1	19	95	...	...	...
2	Feb. 21....	200	0	0	...	...	...	...	...	...	10	90	90	...	...	...
	Feb. 25....	500	0	0	...	...	...	...	...	...	100	400	80	0	300	100
3	Feb. 21....	100	0	0	...	...	...	...	...	...	50	50	50	0	100	100
	Feb. 25....	100	0	0	...	...	...	...	...	...	250	250	50	0	100	100
4	Feb. 21....	100	0	0	...	...	...	...	...	...	25	75	75	0	100	100
	Feb. 25....	100	0	0	...	...	...	...	...	...	150	450	75	0	100	100
5	April 13...	100	0	0	...	...	...	...	...	...	10	115	92	...	...	...
	April 16...	300	0	0	...	...	...	...	...	...	15	285	95	...	...	...
6	April 13...	200	0	0	...	...	...	...	...	...	2	20	90	...	...	...
	April 16...	250	0	0	...	...	...	...	...	...	9	291	97	...	...	...
7	April 13...	175	0	0	...	...	...	...	...	...	14	86	86	...	...	...
	April 16...	150	0	0	...	...	...	...	...	...	30	270	90	...	...	...
8	April 13...	100	0	0	...	...	...	...	...	...	13	91	87	...	...	...
	April 16...	300	0	0	...	...	...	...	...	...	30	270	90	...	...	...
9	April 13...	230	0	0	...	...	...	...	...	...	9	103	91	...	...	...
	April 16...	270	0	0	...	...	...	...	...	...	20	380	95	...	...	...
10	April 13...	300	0	0	...	...	...	...	...	...	2	134	98	...	...	...
	April 16...	300	0	0	...	...	...	...	...	...	2	500	99	...	...	...
	Total.....	5,967	3	.005	178	22	11	7	393	98+	742	4,229	85+	0	700	100

females carry the large additional posterior lateral spines but their other two posterior spines as well as their anterior spines were considerably larger in size than those of the control females.

Cultures containing ten drops of sodium silicate were very difficult to produce and consequently the majority of the experiments were carried on with jars containing five drops. Table I. shows the general results of the experiments and Figs. 1 and 2 show the dorsal views of the females from the control jars contrasted with those from the jars containing sodium silicate. The outline of the skeleton of each female was drawn with the camera lucida with the same magnification.

TABLE II.

SHOWING THAT THE FEMALES WHICH POSSESS THE TWO ADDITIONAL POSTERIOR LATERAL SPINES IN THE CULTURE MEDIUM CONTAINING SODIUM SILICATE WHEN TRANSFERRED TO CULTURE MEDIUM FREE FROM SODIUM SILICATE DO NOT TRANSMIT THE SPINES TO THEIR DESCENDANTS.

Experiments.	Observations, 1916.	Females with Spines transferred from a Jar Containing 5 Drops of Sodium Silicate in 150 c.c. of Culture Medium to a Jar of Culture Medium Free from Sodium Silicate on Feb. 22.		
		Offspring.		
		Females — Spines.	Females + Spines.	Females + Spines 5.
1	Feb. 28.....	99	1	1
2	Feb. 28.....	95	5	5
3	Feb. 28.....	300	0	0

When the females of the *Brachionus amphiceros* type possessing the posterior lateral spines were transferred to jars of culture medium free from sodium silicate the descendants of these females did not develop the lateral spines but developed into the *Brachionus pala* type. Table II shows the results of such experiments.

Whether the rotifers were able to take the silicate out of the water in solution or whether the green flagellates first took it up and then the rotifers eating them obtained the silicate for their additional spines is not certain. A few experiments were performed in an attempt to determine this point. Green *Chlamydomonas* were reared in 150 c.c. culture medium containing 5–7 drops of sodium silicate. When the flagellates had become very numerous and supposedly had taken up as much of the sodium silicate as was possible they were removed from the siliceous medium and put into fresh medium free from sodium silicate. Several dozen rotifers lacking the posterior lateral spines were added. Eight days later two thousand females were

observed and only one possessed the posterior lateral spines. If the *Chlamydomonas* contained sodium silicate the rotifers in using them for food ought to have obtained enough of it with which to have formed the additional spines. When, however, the *Chlamydomonas* were transferred from the siliceous medium to the medium free from sodium silicate the siliceous materials which their bodies may have contained may have diffused out into the general medium, thus leaving their bodies practically free from the siliceous materials and consequently having no effect upon the rotifers.

It is, of course, quite possible that the siliceous material is not used at all in the formation of new spines and the increase in size of the other spines but that the sodium silicate, in some unknown way, stimulates each organism so that it takes out of the water more skeletogenous building material of another kind than under normal conditions. As yet, it is not known that the skeleton contains any siliceous material but it is considered to be entirely of chitinous material. If this is true the effect of sodium silicate upon the rotifers in causing an increase in the number and size of their spines is certainly not clear.

The fertilized eggs that were produced by the spined females of the *Brachionus amphiceros* type in the cultures containing sodium silicate produced females of the *Brachionus pala* type entirely lacking the lateral posterior spines of their mothers. 278 fertilized eggs from such mothers were allowed to develop and none of the females that they produced possessed the additional spines. Sachse and Powers found also that the fertilized eggs of the polymorphic females which they observed always developed into females of one particular type.

Summary.

1. Socium silicate added to the culture medium in which the rotifer *Brachionus pala* was living caused nearly all and in many cases caused all of the descendants to form two additional posterior lateral spines and also to increase the size of the other spines thus forming *Brachionus amphiceros*.

2. The parthenogenetic eggs from females reared in sodium silicate solutions developed into young females of the *Brachionus*

amphiceros type possessing these two lateral spines while the fertilized eggs from females reared in sodium silicate solutions developed into young females of the *Brachionus pala* type lacking these two lateral spines.

3. The variety or species of rotifer, *Brachionus amphiceros*, does not exist as a true variety or species, but is only one of the polymorphic stages of *Brachionus pala*.

4. Perhaps the polymorphic forms of this rotifer as it occurs in pools and ponds during the year are produced by the varying amounts of soluble siliceous substances in the water.

5. The females of *Brachionus amphiceros* possessing the two lateral posterior spines readily produced female parthenogenetic eggs, male parthenogenetic eggs, or fertilized eggs according to the supply of the green food *Chlamydomonas*.

Biological Laboratory,
Wesleyan University,
Middletown, Conn.
April 26, 1916.

LITERATURE CITED.

Langhans, Victor.

'06 Asplanchna priodonta Gosse und ihre Variation. Arch. Hydrobiol. Planktonk., Bd. 1, pp. 439–68.

Lauterborn, R.

'98 Vorläufige Mitteilungen über den Variationskreis von Anuraea cochlearis Gosse. Zool. Anzeiger, Bd. 21, pp. 597–604.

'04 Die cyklische oder temporale Variation von Anuraea cochlearis. Vehr. Nat.-Med. Ver. Heidelberg, N.F., Bd. 7, pp. 529–621.

Mitchell, C. W.

'13 Experimentally Induced Transitions in the Morphological Characters of Asplanchna amphora Hudson, together with Remarks on Sexual Reproduction. Journ. Exper. Zool., vol. 15.

Powers, J. H.

'12 A Case of Polymorphism in Asplanchna Simulating a Mutation. Am. Nat., vol. 46.

Sachse, R.

'12 Biologische Untersuchungen an Rädertieren in Teichgewässern. Sonderabdruck aus Internationale Revue der gesamten Hydrobiol. und Hydrographh.

von Wesenberg-Lund, C.

'98 Über dänishche Rotiferen und über die Portpflanzunggsverhältnisse der Rotiferen. Zool. Anzeiger, Bd. 21, p. 200.

'10 Grundzüge der Biologie und Geographie des Süswasser-Planktons. Sonderabdruck aus Internat. Rev. d. gesam. Hydrobiol. u. Hydrograph.

RELATION OF THE TRUE NUCLEOLUS TO THE LININ NETWORK IN THE GROWTH PERIOD OF PSELLIODES CINCTUS.[1]

WILLIAM M. GOLDSMITH.

(THIRTY FIGURES; TWO PLATES.)

INTRODUCTION.

The purpose of the present line of investigation is to follow the nucleolus in its relation to the other parts of the cell. Except for the extended review and observations of Montgomery ('98), the work on the true nucleolus is scattered in small bits throughout the cytological literature from the first observations of Frontana (1781) to the present time. Especially since the discovery of the sex-chromosomes has the literature on this subject been of a fragmentary character, consisting usually of a few records here and there in papers dealing with other subjects.

The work on the true nucleolus has been greatly retarded by the similarity of the staining reactions of different nuclear bodies and also by the confusion of terms which various observers have used to indicate these nuclear bodies which have an affinity for acid tar-colors and other plasma stains. Ogata ('83) was the first to use the term "plasmosome" with reference to the true nucleolus. The term "paranuclein" of O. Hertwig ('92) and "pyrenin" of Schwartz ('87) are ofttimes erroneously applied to the plasmosome itself, rather than to the substance of the same. Eisen ('98) used the term "linoplast," since he insists that the function of this body is to supply and nourish the linin network during mitosis. To prevent further confusion, I shall use the older and more familiar term, "nucleolus." The term "nucleolus" however carries with it no definite meaning and thus should not be used without a modifying adjective, either expressed or implied, as the cytological literature contains many errors and misconceptions on account of the lack of a clear dis-

[1] Contribution No. 152 from the Department of Zoölogy, Indiana University.

tinction between the various kinds of nucleoli. This difficulty renders it almost impossible to present a satisfactory review of the literature of the plasmosome, as so many writers discuss the "nucleoli" when even the context fails to indicate the kind of nucleoli under consideration. Carnoy ('85) finds four distinct kinds, namely, "nucléoles nucléiniens, nucléoles plasmatiques, nucléoles mixtes, and nucléoles noyaux." It seems advisable, however, to adhere closely to the well-known broader classification (chromatic and achromatic nucleoli) and always endeavor to make the distinction very clear.

Observations.[1]

It would be very desirable to have at hand a large number of species which show the points under consideration. As this is impossible at this time, I shall present the results based upon a study of *Pselliodes* (*milyas*) *cinctus* Fabr.

The testes of *Pselliodes cinctus* are composed of cylindrical but tapering follicles which are wound about in a very irregular form. Each follicle contains definite walled cysts, but on account of this winding shape it is very unusual to obtain even a longitudinal section which shows more than a half dozen cysts in the same follicle. This difficulty, however, is offset by the fact that the follicles show a regularity in the zonation of the various stages of development from one end to the other. Even though many stages of growth may be omitted, no cyst has been found containing cells which are in a more advanced stage than those further down the follicle. This makes it a comparatively easy matter to obtain the sequence of stages in development.

Since it is primarily beyond the scope of this paper to deal with the chromatic parts of the cell, except so far as related directly to the development of the achromatic parts of the nucleolus, the figures include only the stages of the growth period between the synaptic stage and the first spermatocyte division. Incidentally, however, observations were made upon the stages of spermatogenesis both preceding the appearance and following the disappearance of the plasmosome material. The periods in

[1] While working on the sex-chromosomes in the Reduviidæ, Dr. F. Payne made observations which enabled him to suggest the present line of research.

maturation prior to the synaptic stage correspond in general to those of *Oncopeltus* as given by Wilson ('12). It was further noted, in agreement with the observations of Payne ('12), that the oögonial number of chromosomes is thirty, the spermatogonial twenty-eight, and the first spermatocyte sixteen. The metaphase plate of the second maturation division shows twelve chromosomes arranged in a more or less uniform circle surrounding four (three small and one large) sex-chromosomes.

A. Origin, Development and Fate of the Nucleolus.

The contraction stage presented in Fig. 1 is of considerable duration. As this figure shows, the chromatin threads are thick and stain intensely. The threads even though in a contracted condition, exhibit a certain degree of polarization, many of the fibers terminating in the vicinity of the nucleolus, but seldom if ever coming in contact with it.

Embedded in the periphery of this mass of chromatin threads lies the large, compact, well-defined nucleolus surrounded by a vacuole-like space as described and illustrated by Wilson ('12) in *Largus* and other forms. As he says of *Largus* (Figs. 78 to 80), "no definite wall to the vacuole can be seen, but the chromatin threads are often seen encircling its outer limit as if lying upon a definite substratum." This noteworthy feature is characteristic of all the preceding stages back to the telophase of the last spermatogonial division. However, in the earlier stages the body is somewhat smaller and more spherical in form. This slight increase in size and the change of shape suggests a certain degree of activity prior to the stage represented in Fig. 1. After this period, however, the growth is so rapid that seldom can two adjacent cysts be found which show the same stage of nucleolar development.

Under ordinary staining conditions the nucleolus shows no differentiation in the structure until after the nuclear wall has formed (Fig. 15). In fact the condition was so universal that up to this stage the nucleolar body was first considered solely chromatic in nature. Later observations, however, revealed the fact that *the nucleolus is not a homogeneous body but composed of both chromatic and achromatic material.* This conclusion is based

upon a study of the development of this structure and its reaction to stains. The earliest possible differentiation that can be observed is found immediately following the synaptic stage (Figs. 5 and 6). The nucleolus at this time is of a more or less irregular shape, and sometimes certain parts of it take the stain less intensely. The more deeply staining areas give the appearance of irregular granular masses. Although later development shows that these masses are the sex-chromosomes in a diffused condition, they are in this early stage poorly defined (Figs. 12 and 13).

As the cell continues to grow, a gradual growth in the nucleolus is likewise noted. The distinction between the chromatin and the achromatin becomes more noticeable as the chromatin aggregations gradually condense. The condensation of this chromatin material causes certain areas of the nucleolus to become clearer, or to take the plasma stain in case a counter stain is used. Many instances are noted in which one end of a chromatin body is much more compact than the other (Figs. 13 and 16), in which case the corresponding achromatic part of the nucleolus is much more homogeneous.

Another noteworthy feature, characteristic of these stages in the early life of the nucleolus, is the irregular contour. In practically every nucleolus the more granular and loose in texture the chromatic areas are, the more irregular is the whole body. For example, Fig. 14 shows various evaginations of the plasma material in which are found chromatin-granules either in a loose (*d*) or compact (*a*) condition. After the chromatin granules are condensed into four compact bodies, the sex-chromosomes, the whole mass presents a more globular appearance (Figs. 17, 18, 19 and 20). Fig. 18 is a typical figure of this stage. The condition shown in Fig. 17 is seldom observed, while only three or four cells were found which show four clear-cut chromosomes in the same plane (Fig. 20). There is nothing problematical regarding this change to a uniform spherical shape, as the withdrawal of the chromatin granules leaves the achromatic portion of the nucleolus less dense and thus gives it an opportunity to assume a more globular form, in accordance with the general laws of fluid action. In the previous stages the

amount of plasma substance is so small in comparison with the embedded chromatin, that the latter acts as the governing factor in determining the shape of the whole mass; but by the time the stage under consideration (Figs. 17 to 20) is reached, the achromatic part of the nucleolus is sufficiently large to be only slightly affected by the presence of the embedded bodies. However, in cases where the nucleolus has a slight irregular contour, the same can usually be accounted for by the presence of one or more of the sex-chromosomes near the periphery.

The nucleus now has a period of very rapid growth accompanied by noticeable extra-nucleolar changes. In the earlier stages the nucleus presents a somewhat uniform fibrillar but granular appearance, though much darker near the nuclear membrane than in the central part. The clear area around the nucleolus increases in size and, in the middle growth period, shows no traces of linin. As the cell continues to grow the linin becomes more compact, first near the nuclear wall and then nearer and nearer the nucleolus (Figs. 16 to 20). Numerous instances may be seen where entanglements of linin are scattered indiscriminately throughout the nucleus even into the vacuole-like space.

At a little later stage this whole complex mass between the nucleolus and nuclear wall, presents the appearance of innumerable anastomosing threads of a fibrillar substance, in which are embedded minute granules. These fibers continue to elongate until they come in contact and ultimately fuse with the true nucleolar portion of the nucleolus. This fusion, together with a continued growth on the part of both the true nucleolus and the surrounding linin, causes the two to become continuous and to assume a similar appearance (Figs. 22 and 23). Prior to this time the achromatic part of the nucleolus has presented a typical homogeneous appearance, but as more granular fibers come in contact and fuse with it, it assumes a more granular appearance (Figs. 21–25). The achromatic part of the nucleolus now greatly resembles the granular appearance shown in the stages prior to the individualizing of the sex-chromosomes. This condition suggests that chromatin granules have moved along the linin and entered the true nucleolus. *This indicates that the plasmo-*

some is linin or closely related in composition to it. As the linin and the achromatic body becomes continuous the latter is pulled into various shapes and becomes practically indistinguishable from the linin (Fig. 25). While these changes are taking place, the peripheral areas of linin become more conspicuous—the larger number of these aggregations being either near or in contact with the nuclear membrane. In order to form the chromosomes the linin collects, leaving wide intervening spaces almost clear (Figs. 23–26). Usually in the formation of these aggregations, strands of linin connecting the centralized mass (the former nucleolus) and the nuclear membrane remain in contact on one side longer than on the other. Whether or not this action is associated with the movement of the central mass to the vicinity of the nuclear wall in early prophase, we can only surmise (Figs. 25 and 26). In the meantime the achromatic parts of the nucleolus and the linin network, become more and more indistinguishable from one another.

As the aggregations of chromatin and linin become more compact, in the formation of the bivalent chromosomes of the first spermatocyte division (Figs. 28 to 30), masses and strands of achromatin may be observed attached to the chromosomes and floating in the cell sap (Fig. 27). This same condition is figured by Stevens ('11) in the spermatocyte cells of the guinea pig. She notes that "in early prophase the chromatin appears as though gathering together about definite centers along the spireme, leaving the linin threads between." However, the chromosomes in the material under consideration, are so far apart that the strands of linin are usually broken between them. By this time, no discrimination can be made between the achromatic part of the former nucleolus and the linin, except in a few cases where large collections of achromatic material is indicative of the former (Fig. 28).

Discussion.

It would be next to impossible to draw conclusions of general application from the above observations, since they are based upon only one species. It seems advisable, however, to call attention to some of the important facts regarding the origin,

development, fate, and function of the nucleolus of *Pselliodes*, and to compare these observations with those of a few other workers who have incidentally noted facts along the same line.

Origin.—Since the chromatin and achromatin of the nucleolus are indistinguishable in the early growth period, it is impossible to follow the achromatic material back to its first appearance. My observations, however, clearly show that its origin is in some way related to the chromatin. This suggestion does not corroborate the views of Montgomery and many earlier workers, but finds support in the observations of a large number of later investigators. The theory of the extra nuclear origin of the achromatic nucleolus, as set forth by Korschelt ('89) and strongly supported by Montgomery ('98) readily loses its significance, at least in general application, when the life history of the nucleolus of *Pselliodes* and similar forms are understood. The only proof set forth in support of this view is the fact that such nucleoli first appear in contact with the nuclear membrane. In many forms, the true nucleolus not only originates independent of, but completes its life history without being in any way associated with the nuclear membrane. Medes ('04), for example, notes that in the growth period of *Scutigera forceps* the chromatin accumulates, forming a karyosphere which seems to be of an achromatic nature, although containing the chromatin in granular form. The granules finally emerge leaving an achromatic mass, containing an accessory chromosome. The achromatic material now breaks into small rounded bodies, which soon become indistinguishable. The true nucleolus of *Philosamia cynthia* (Dederer, '07) also appears in a mass of chromatin entangled in the spireme threads of the last spermatogonial stage. In this and later stages, it is characteristically bipartite, one end of which usually stains darker, no doubt from the fact that it contains more chromatin granules. In its typical form, the idiochromosomes are attached around the middle of the double achromatic nucleolus in the form of a deep crescent-shaped band. This band later becomes shorter and broader until it loses contact with the nucleolus, which then disappears.

Although these citations will suffice to show that in many forms there is a certain uniformity in the early history of the

nucleolus, I wish to refer to the fact that many observers have found the sex-chromosomes either imbedded in, or associated with an achromatic nucleolus, as considered in detail in *Pselliodes*. Although the writers referred to below did not follow in detail the earlier history of the achromatic material many of their illustrations and descriptions suggest a uniformity. Payne ('09) finds the sex chromosomes of a number of Reduviidæ embedded in a plasmosome-like body as shown in *Pselliodes* (Figs. 17–19). He notes an achromatic nucleolus and a chromatic body (later the differential chromosomes) in the synaptic stage of *Prionidus cristatus*. After this stage the achromatic material forms around the chromatic body, and by fusion of the two bodies, a condition similar to figure 20 is assumed. Wilson ('05) figures a close relationship between the plasmosome and idiochromosomes of *Lygæus* and *Brochymena*. Boring ('07) notes a true nucleolus associated with an odd chromosome in *Aphrophora quadrangularis;* while in the blue rove beetle, *Staphylinus violaceus* (Stevens '08), and the earwig, *Anisolabis martima* (Randolph '08), this body is associated with a heterochromosome. Stevens ('08) notes that, "in the growth stage [of *Calliphora vomitoria*] the hetero-chromosomes are associated with a plasmosome as in many species of Coleoptera." Her Fig. 11 is suggestive of the condition found in the early history of the nucleolus of *Pselliodes*.

The direct relation which exists between the chromatin and the achromatin, clearly accounts for the erroneous theories of the earlier workers regarding the origin of the true and chromatin nucleolus. Montgomery ('98) states that according to his observations the true nucleolus is never derived from the chromatin. King ('08) notes that the achromatic body (in *Bufo lentiginosus*) is either embedded in the chromatin granules or surrounded by balls of chromatin attached to the surface, and further that "there is nothing to indicate that the chromatin in these structures (karyospheres) is derived from the plasmosome or vice versa." Ruzicka ('06) considers the true nucleolus as an intermediate stage between the chromatin and the linin. *My own observations have led me to conclude that in Pselliodes the true nucleolus is formed by the accumulation of linin about the sex-chromosomes.*

Development and Fate.—The observations on the development and disappearance of the plasmosome in *Pselliodes* seem to find agreement in many other forms. The observations of Medes ('04) on *Scutigera* and of Dederer ('07) on *Philosamia* are representative types of this agreement.

The latter part of the life history of the nucleolus, in which this body is again assuming a granular texture, is suggestive of that of *Culex* (Stevens '10), in which the achromatic body absorbs chromatin substance extruded from the spireme during the synizesis stage. Stevens bases her conclusion on the fact that the body presents a series of colorations in the growth period. She continues: "Whether the rejected material [from chromatin], visible in some cases, is waste material or substances which have some function connected with the growth stage of the germ cell, we can only surmise." No doubt, *Pselliodes* presents better material for a study of this point, as the foregoing observations clearly show that this chromatin is used in the formation of the first spermatocyte chromosomes (Figs. 26 to 30).

The later stages in the life of the nucleolus, in *Pselliodes*, lead to a suggestion as to its nature. It will be remembered that the linin becomes attached to various parts of this spherical body. The achromatic part then assumes a fibrillar but granular appearance, and becomes indistinguishable from the linin. These facts and observations, together with the recognized nature and functions of the linin, lead me to suggest that *the plasmosome and linin not only possess similar characters, but are one and the same material, the former being simply a globular mass of the latter.*

Function.—Since the achromatic part of the nucleolus of *Pselliodes* seems to be a modified form of the linin, we should expect a certain similarity in the functions of each. Further, since the function of the linin is to support the chromatin during the various stages of cell activity, we would attempt to ascribe a similar function to the plasmosome. A brief review of the preceding observations and illustrations clearly shows that we are not disappointed in the attempt. In *Pselliodes* the behavior of the sex-chromosomes and the achromatic material is identical with the corresponding action of the chromatin and linin. The

compact chromatin nucleolus of the early growth period takes up particles of achromatic material, and as the chromatic material assumes a less compact form, more achromatin (linin) is added, seemingly to act as a support to the chromatin aggregations. This growth continues until the sex-chromosomes are well differentiated and the true nucleolar body reaches its maximum size. *This behavior, as well as the method of breaking down of the achromatic material, indicates that the plasmosome has the same function that has been ascribed to the linin, namely, to support the chromatin material.*

B. Granules and Nuclear Membrane Formation.

In addition to the origin and early development of the nucleolus, the first thirteen drawings are intended to show the apparent relation that exists between certain granules and the formation of the nuclear membrane. The facts involved in this additional observation are not directly related to the problem in hand, but since they are presented in the same series of illustrations, a discussion of them does not seem out of place.

As was suggested in the preceding discussion, the chromatin fibers of the synaptic stage (Figs. 1 and 2) are thick and stain intensely. These threads, though in a contracted condition, possess a number of granular, knob-like enlargements, which stain more intensely than do the remaining parts of the thread. After the threads remain in this condition for some time, they elongate and decrease in thickness (Figs. 3, 4 and 6).

By virtue of this elongation, the chromatin mass becomes less compact and thus increases in size. At the stage represented in Fig. 4, the bead-like enlargements on the threads, though smaller and more compact, have become more defined, still retaining the stain. Thus they become somewhat individualized, while the part of the thread intervening between the beads takes the chromatin stain less intensely. This gives the thread the appearance of a number of granules (perhaps chromomeres) held in place by some substance quite dense in consistency. In fact, many chromatin fibers were found in this and subsequent stages, in which the contrast was so great between the deeply staining granular enlargements and the intervening spaces, that the

whole thread presented the appearance of a row of granular bodies with only here and there a fibrous connection (Figs. 7 and 8).

As the chromatin threads begin to elongate after the synaptic stage, the chromatic area as a whole begins gradually to stain less intensely until the stage represented in Fig. 7 is reached, when, in good preparations, nothing can be observed except the large nucleolus, surrounded by faint traces of fibers and a large number of granules. This gradual decrease in the staining capacity of the chromatin material rendered the tracing of the various transitional stages very difficult. Although the evidence was not entirely conclusive, the most careful observations suggested that the granules found in this and later stages were formerly a part of the chromatin threads and are distinctly chromatic in nature—perhaps being either a part of the true chromosomes or directly related to them.

Throughout the early growth period, no traces of a nuclear wall has been observed. In many and perhaps the majority of cells, the chromatin threads and granules seem to have a definite limiting space. However, among the same and in other cells, innumerable instances can be found in which the granules and granular rows are continuous far beyond what could be considered the nuclear area (Figs. 8 and 9), thus indicating the non-existence of a nuclear membrane. Flemming in the blood cells of Amphibians, Hertwig in the sperm-mother-cells of Nematodes, and other more recent workers have referred to the difficulty involved in the demonstration of the presence of a nuclear membrane in certain stages of cell growth.

The granules are now found scattered indiscriminately throughout the entire cell. The natural growth of the nucleolus and a simultaneous enlargement of the surrounding clear area, causes these granules to be crowded together between the nucleolus and the cell wall, forming a more or less incomplete circle around the nuclear area (Figs. 10 and 11). As more and more granules are added to the circle, visible strands of linin can be noted between them. The next stages (Figs. 11, 12 and 13) are characterized by a gradual decrease in staining capacity of the larger granules, simultaneously with a continued increase of the inter-

vening fibrillar bridges, until the nuclear wall becomes visible and presents a homogeneous appearance (Figs. 15, 16, etc.). This membrane, though invisible prior to this time, remains very conspicuous until the prophase of the first spermatocyte division. *These observations, while not conclusive, indicate that the nuclear membrane may be, at least in part, chromatic.*

LITERATURE CITED.

Boring, Alice M.

'07 A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ, with Special Reference to the Behavior of the Odd Chromosome. Jour. Exp. Zool., Vol. 4.

Carnoy, J. B.

'85 La Cytodiérése chez les Arthropodes. La Cellule, T. I.

Dederer, Pauline H.

'07 Spermatogenesis in Philosamia cynthia. BIOL. BULL., Vol. 13.

Eisen, Gustav.

'00 Spermatogenesis of Batrachoseps. Jour. Morph., Vol. 17.

King, H. D.

'08 The Oögenesis of Bufo lentiginosus. Jour. Morph., Vol. 19.

McClendon, J. F.

'07 The Spermatogenesis of Pandarus sinnatus Say. BIOL. BULL., Vol. 13.

Medes, Grace.

'04 The Spermatogenesis of Scutigera forceps. BIOL. BULL., Vol. 9.

Montgomery, Thos. H.

'98 Comparative Cytological Studies, with Especial Regard to the Morphology of the Nucleolus. Jour. Morph., Vol. 15.

Payne, Fernandus.

'09 Some New Types of Chromosome Distribution and their Relation to Sex. BIOL. BULL., Vol. 16.

'12 A Further Study of the Chromosomes of the Reduviidæ. Jour. Morph., Vol. 23.

Randolph, Harriet.

'08 The Spermatogenesis of the Ear-wig, Anisolabis martima. BIOL. BULL., Vol. 15.

Ruzicka, Vladislav.

'06 Struktur und Plasma. Ergebnisse der Anatomie und Entwickelungegeschichte, Band 16.

Stevens, N. M.

'08 A Study of the Germ Cells of Certain Diptera, with Reference to the Hetero-chromosomes and the Phenomena of Synapsis. Jour. Exp. Zool., Vol. 5.

'09 Further Studies on the Chromosomes of the Coleoptera. Jour. Exp. Zool., Vol. 6.

'10 The Chromosomes in the Germ Cells of Culex. Jour. Exp. Zool., Vol. 8.

'11 Hetero-chromosomes in the Guinea Pig. BIOL. BULL., Vol. 21.

Wilson, E. B.

'12 Studies on Chromosomes. Observations on the Maturation Phenomena in Certain Hemiptera and Other Forms, with Consideration on Synapsis and Reduction. Jour. Exp. Zool., Vol. 13.

'05 Studies on Chromosomes. The Behavior of the Idiochromosomes in Hemiptera. Jour. Exp. Zool., Vol. 2.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Contraction stage with chromatic fibers bearing granular enlargements. The nucleolus is shown in this, and the three succeeding figures as a homogeneous body.

FIGS. 2 AND 3. Chromatin threads emerging from the contraction stage.

FIGS. 4 AND 5. Further elongation of the chromatin threads. The enlargements have become smaller but more compact.

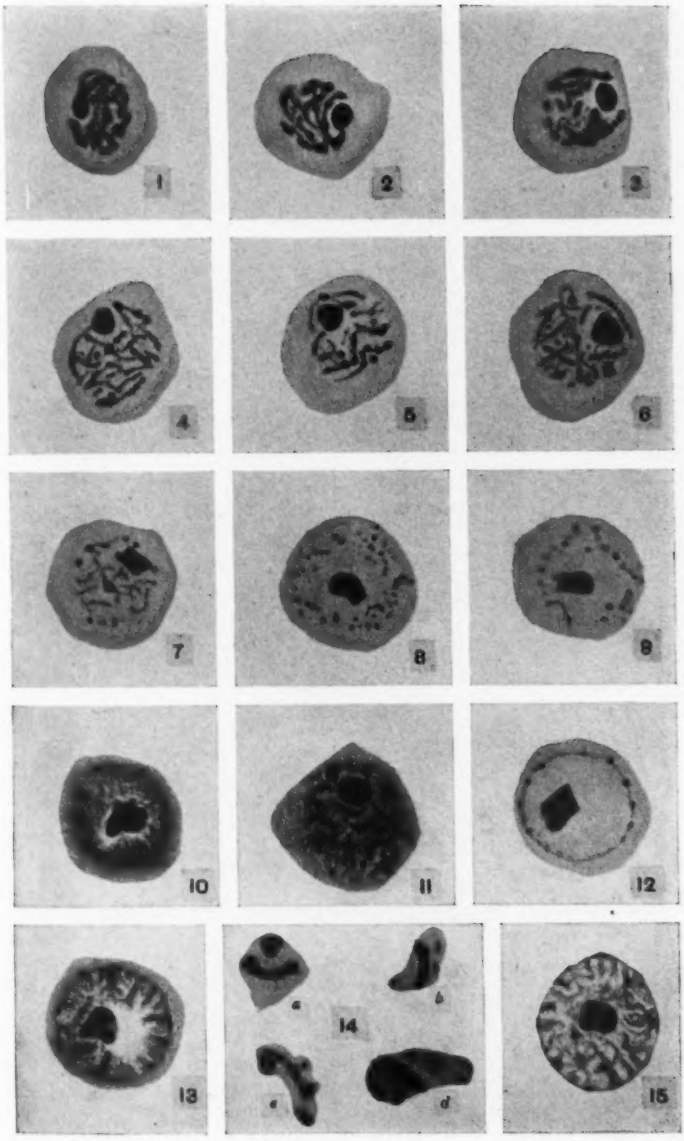
FIGS. 4 TO 17. Successive stages in the formation of the nucleolus.

FIGS. 6 AND 7. Individualizing the granules found in succeeding stages. Nucleolus shows its achromatic nature.

FIGS. 8 TO 13. Stages in the formation of the nuclear wall. Further differentiation of the chromatin and achromatin.

FIG. 14. Various shapes assumed by the chromatic and achromatic substance of the nucleoli.

FIGS. 15 AND 16. Nuclei showing further development of the nucleolus.



WILLIAM M. GOLDSMITH.

PLATE II.

FIGS. 17 TO 20. Nuclei with nucleoli made up of achromatin and the sex-chromosomes. Linin approaching the nucleoli.

FIGS. 20 TO 29. Successive stages in the disappearance of the nucleolus.

FIG. 21. Fusion of achromatic part of the nucleolus and linin.

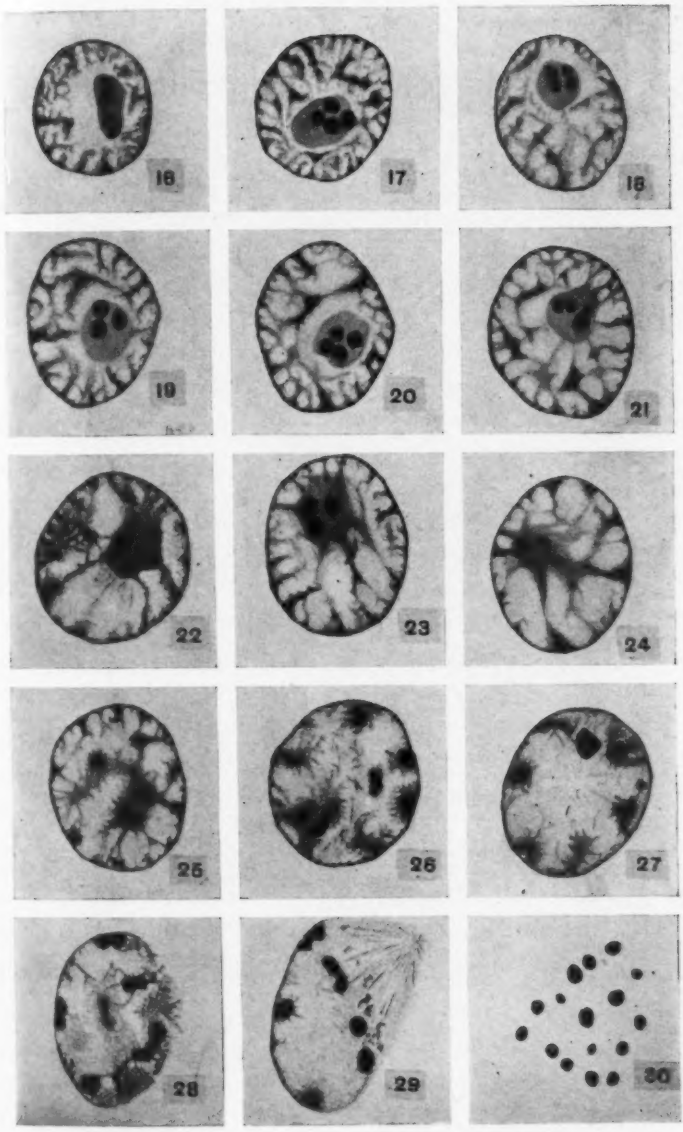
FIGS. 22 TO 25. Successive stages in the fusion of the true nucleolus and linin, the former becoming indistinguishable from the latter.

FIGS. 25 TO 30. Formation of the prophase chromosomes for the first spermatocyte division.

FIGS. 26 AND 27. Remains of the old nucleolus being drawn to the nuclear membrane.

FIGS. 28 AND 29. Breaking down the nuclear membrane by the formation of the first spermatocyte spindle.

FIG. 30. Metaphase plate of the first spermatocyte division.


16
17
18
19
20
21
22
23
24
25
26
27
28
29
30